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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12Q 1/68, G06F 17/00, 7/00, 17/30

A1

(11) International Publication Number: **WO 00/39338**

(43) International Publication Date: 6 July 2000 (06.07.00)

(21) International Application Number: PCT/US99/30643

(22) International Filing Date: 22 December 1999 (22.12.99)

(30) Priority Data:
09/220,276 23 December 1998 (23.12.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(54) Title: METHOD AND SYSTEM FOR ANALYZING BIOLOGICAL RESPONSE SIGNAL DATA

(57) Abstract

A system, method, and computer program product for enhanced computer–aided analysis of biological response data is disclosed. In a preferred embodiment, biological datasets are graphically selected by a user from a first active biological viewer window on a user computer display and projected onto one or more other active biological viewers on the display. The selected data is highlighted in the destination biological viewers using contrast or color differentiation from other data appearing in the destination windows. In another preferred embodiment, hierarchical cluster trees from biological signal profiles are presented in a hyperbolic display fashion. In another preferred embodiment, biological menu and submenu items utilized by the user computer are not stored in the user computer, but rather are stored in a central biological response database. Biological menus and submenus are generated at startup time based on queries to the central biological response database, allowing for increased flexibility, changeability, and customization of the biological menus. In another preferred embodiment, correlation data between biological signal profile data is precomputed when the experiments are added to the central biological response database, eliminating the need for real time computation of correlation coefficients by the user computer.

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# METHOD AND SYSTEM FOR ANALYZING BIOLOGICAL RESPONSE SIGNAL DATA

This is a continuation-in-part of copending application no. 09/220,276 by Bassett *et al.* filed December 23, 1998 entitled "Method and System For Analyzing Biological Response Signal Data," which is incorporated by reference herein in its entirety.

## 1. FIELD OF THE INVENTION

The present invention relates to the field of computer-assisted analysis of biological information. In particular, the present invention relates to a method and system for management of a database containing biological response signal data and for presentation of useful analytical displays of information therefrom.

## 2. BACKGROUND OF THE INVENTION

The analysis of complex systems such as biological organisms is aided by the use of relational database systems for storing and retrieving large amounts of biological data. The advent of high-speed wide area networks and the Internet, together with the client/server based model of relational database management systems, is particularly well-suited for allowing researchers to access and meaningfully analyze large amounts of biological data given the appropriate hardware and software computing tools.

Computerized analysis tools are particularly useful in experimental environments involving biological response signals. By way of nonlimiting example, biological response signal data can be obtained and/or gathered using biological response signal matrices, that is, physical matrices of biological material that transmit machine-readable signals corresponding to biological content or activity at each site in the matrix. In these systems, responses to biological or environmental stimuli may be measured and analyzed in a large-scale fashion through computer-based scanning of the machine-readable signals, *e.g.* photons or electrical signals, into numerical matrices, and through the storage of the numerical data into relational databases.

As a further nonlimiting example, biological response signal data can be obtained and/or gathered using serial analysis of gene expression (SAGE) or other technologies for measuring gene/protein expression levels that may not use a matrix or microarray but otherwise produce measurable signals. Generally speaking, biological response signals may be measured after a perturbation of a biological sample including, for example, the exposure of a biological sample to a drug candidate, the introduction of an exogenous gene into a

biological sample, the deletion of a gene from the biological sample, or changes in the culture conditions of the biological sample.

A useful outcome of the scientific experimentation being performed involves the understanding of the relationships between genes and perturbations, understanding that promotes other useful outcomes such as the invention of new drugs or other therapies. Often, relationships between perturbation and gene expression levels sheds light on known or unknown biological pathways. There is an ongoing need in the art to generate better and more useful ways for computers to assist in analyzing the large volume of biological response data that can exist for even the most simple biological organisms.

## 3. SUMMARY OF THE INVENTION

A system, method, and computer program product are provided for improved computer-aided analysis of biological data derived from machine readable outputs of experiments performed on a plurality of biological samples. Responsive to search and execution commands from the user, a plurality of biological viewer windows are spawned on a user computer display. The user may then select a source dataset from one of the biological viewers and execute a project selection command, wherein the source dataset is then projected onto the other biological viewers. The projections are characterized by a highlighted display of biological data points in the destination biological viewers corresponding to items in the source dataset. The selected data is highlighted in the destination biological viewers using contrast or color differentiation from other destination window data.

In another preferred embodiment, the user may spawn a hierarchical cluster tree biological viewer that displays genes or experiments grouped based on similarity of behavior, wherein the hierarchical cluster tree is displayed in a hyperbolic display fashion. In one form, the hierarchical cluster tree may be, for example, a gene coregulation tree. When displayed in a hyperbolic display fashion, convenient viewing of the hierarchical cluster tree is enabled, whereby the user may move around the tree and zoom in and out of various areas of the tree without losing perspective of their current location relative to the "root" of the tree.

In another preferred embodiment, biological menu and submenu items that are displayed to the user during searches, projections, and the like are not stored in the user computer, but rather are stored in a central biological response database. Biological menus and submenus are generated at startup based on queries to the central biological response database, allowing for increased flexibility, changeability, and customization of the biological menus and submenus.

In another preferred embodiment, correlation data between expression array experiments is precomputed when the experiments are added to the central biological response database. This eliminates the need for real time computation of correlation coefficients or other similarity scores by the user computer, resulting in considerable time savings when the user requests correlation data among selected sets of experiments.

Thus, in various embodiments, the invention provides (i) computer systems programmed to carry out the analysis of biological data, (ii) the computer programs themselves, or (iii) the computer programs recorded on a carrier. Methods for validating a drug target by use of the computer program products of the invention are also provided. Computer readable memories comprising instructions for carrying out the analysis of biological data according to the invention are further provided.

In a specific embodiment the invention is directed to a computer program product for directing a user computer in a computer-aided analysis of biological response data, the biological response data being derived from machine readable outputs of biological signal experiments performed on one or more biological samples, said computer program product comprising:

computer code for receiving search commands from a user and displaying information related to said biological response data on a plurality of viewers on a user display;

computer code for receiving a source dataset selection command from the user for identifying a source dataset, said source dataset corresponding to a subset of biological data being displayed on a source viewer, said source viewer being one of said plurality of viewers;

computer code for projecting said source dataset onto a destination viewer, said projection being characterized by a highlighted display of data in said destination viewer that corresponds to said source dataset from said source viewer, said destination viewer being a different one of said plurality of viewers than said source viewer;

whereby user observation of biological relationships that may exist in said biological samples may be enhanced through the facilitated viewing of said source dataset as projected onto said destination viewer.

The invention also provides a computer program product for directing a user computer in a computer-aided analysis of biological signals, the expression array data being derived from machine readable outputs of biological response experiments performed on a plurality of biological samples, said computer program product comprising:

computer code for generating a cluster tree using said expression array data;

and

computer code for displaying said cluster tree in a hyperbolic display format.

In another embodiment, a computer program product is provided for directing a user computer to perform dynamic menu generation for receiving a required biological data field selection from a user, the user computer connected to a central database server across a computer network, said computer program product comprising:

computer code for querying said central database server upon instantiation of said computer program product on said user computer, said query being adapted to request dynamic menu information from said central database server, said dynamic menu information defining biological data field items, subcategory items, and submenuing relationships among said biological data field items and subcategory items;

computer code for displaying a first menu on a user display, said first menu comprising at least a portion of said biological data field items and subcategory items;

computer code for receiving a first menu selection from the user;

computer code for determining whether said first menu selection is a biological data field or a subcategory item;

computer code for identifying said first menu selection as the required biological data field selection if said first menu selection is a biological data field; and

computer code for identifying submenu information contained in said dynamic menu information if said first menu selection is a subcategory item, and for displaying said submenu information on a second menu near said first menu on said user display;

whereby said user computer is not required to store dynamic menu information, said central database server being capable of storing substantially all required dynamic menu information, allowing for enhanced flexibility and customization capability of biological menu systems on said user computer.

Another computer program product provided by the invention is for directing a user computer in a computer-aided analysis of expression array data, the expression array data comprising an image of an expression array, the computer program product comprising:

computer code for computing one or more quality statistics relating to the image;

and

computer code for simultaneously displaying on a computer screen said quality statistics alongside said image.

Another computer program product of the invention is for use in conjunction with a computer system, the computer program product comprising a computer readable storage medium and a computer program mechanism embedded therein, the computer program mechanism comprising:

instructions for displaying biological data on a user display;

instructions for receiving a selection of a subset of said biological data from a user;

instructions for generating a context-sensitive menu in response to said selection, wherein a menu option in said context-sensitive menu comprises a substitution tag;

instructions for obtaining a first menu selection from said user;

instructions for replacing a substitution tag in said first menu selection, said replacing being based on an operation that uses a portion of said subset of said biological data, thereby building a substituted menu option; and

instructions for executing said substituted menu option.

Among the computer readable memories provided by the invention is a computer readable memory used to direct a computer to function in a specified manner, said memory comprising a module capable of analyzing biological data; wherein said module comprises:

instructions for displaying biological data on a user display;

instructions for receiving a selection of a subset of said biological data from a user;

instructions for generating a context-sensitive menu in response to said selection, wherein a menu option in said context-sensitive menu comprises a substitution tag;

instructions for obtaining a first menu selection from said user;

instructions for replacing a substitution tag in said first menu selection, said replacing being based on an operation that uses a portion of said subset of said biological data, thereby building a substituted menu option; and

instructions for executing said substituted menu option.

Also provided is a computer readable memory used to direct a computer to function in a specified manner, said memory comprising a module capable of analyzing biological data; wherein said module comprises:

instructions for receiving search commands from a user and displaying information related to said biological response data on a plurality of viewers on a user display;

instructions for receiving a source dataset selection command from the user for identifying a source dataset, said source dataset corresponding to a subset of

biological data being displayed on a source viewer, said source viewer being one of said plurality of viewers; and

instructions for projecting said source dataset onto a destination viewer, said projection being characterized by a highlighted display of data in said destination viewer that corresponds to said source dataset from said source viewer, said destination viewer being a different one of said plurality of viewers than said source viewer;

whereby user observation of biological relationships that may exist in said biological samples may be enhanced through the facilitated viewing of said source dataset as projected onto said destination viewer.

In a specific embodiment, the invention provides a method for processing biological signal profile data for storage in a central biological database server and for retrieval therefrom by a user computer connected to the central database server across a computer network, comprising the steps of:

storing a plurality of biological signal profiles in said central database server;

computing a correlation metric between at least one of said plurality of biological signal profiles and other of said plurality of biological signal profiles in said central database server;

storing said correlation metrics in said central database server in conjunction with said expression array experiments;

at said user computer, at a time subsequent to said step of storing said correlation metrics, selecting a query profile and a target set of biological response profiles from among said plurality of biological response profiles in said central database server;

receiving correlation criteria and a correlation execution command from a user at said user computer;

querying said central database server to identify biological response profiles between said source set and said target set having correlation metrics that satisfy said correlation criteria;

transmitting information related to said identified biological response profiles and related correlation metrics from said cental biological database server to said user computer;

and

displaying information derived from said biological response profiles and related correlation metrics at said user computer;

whereby real time computation of said correlation metrics is not required at said user computer after said correlation execution command, thereby enhancing speed of biological correlation analysis at said user computer.

In a specific embodiment, the invention provides methods for analyzing expression array data using a computer, the expression array data being derived from machine readable outputs of perturbation experiments performed on a plurality of biological samples. In one embodiment, the method comprises the steps of:

generating a coregulation tree using said expression array data;

displaying said coregulation tree in a hyperbolic display format;

manipulating the display of the coregulation tree to display a first gene or gene product located on a branch thereof, said first gene or gene product having a known function;

locating a second gene or gene product on said coregulation tree on the same branch as said first gene or gene product;

and

assigning a function to said second gene or gene product using information related to said known function of said first gene or gene product and information related to a positional relationship or distance metric between said second gene or gene product and said first gene or gene product on said branch of said coregulation tree.

In another embodiment, the method comprises the steps of:

generating an experiment profile cluster tree using said expression array data;

displaying said experiment profile cluster tree in a hyperbolic display format;

manipulating the display of the experiment profile cluster tree to display a first experiment located on a branch thereof, said first experiment corresponding to a known function of a perturbation assayed therein;

locating a second experiment on said experiment profile cluster tree on the same branch as said first experiment;

and

assigning a function to a biological perturbation assayed in said second experiment using information related to said known function of said perturbation assayed in said first experiment and information related to a positional relationship or distance metric between said second experiment and said first experiment on said branch of said experiment profile cluster tree.

A method of validating a drug target, by use of a computer program product of the invention is also provided, wherein a first set of biological signal response profiles, which are selected from a plurality of biological signal response profiles, is subtracted from a second set of biological signal response profiles, which are selected from a plurality of signal response profiles.

The invention is also directed to a method for generating a context-sensitive menu for use in building and executing a customized menu option that is related to the study of biological information, said method comprising the steps of:

displaying biological data on a user display;

receiving a selection of a subset of said biological data from a user;

generating a context-sensitive menu in response to said selection of said subset of biological data, wherein a menu option in said context-sensitive menu comprises a substitution tag;

obtaining a first menu selection from said user;

replacing a substitution tag in said menu selection choice, said replacing being based on an operation that uses a portion of said subset of said selected biological data, thereby building a substituted menu option; and

executing said substituted menu option; wherein said executed substituted menu option provides useful biological information that is related to said subset of said biological data selected by said user.

## 4. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a biological response data network in accordance with a preferred embodiment;

FIG. 2 shows an image of a DNA microarray;

FIG. 3 shows a block diagram corresponding to three primary software elements of a biological response data network in accordance with a preferred embodiment;

FIG. 4 shows a query composition and results display window in accordance with a preferred embodiment;

FIG. 5 shows a signature plot biological viewer in accordance with a preferred embodiment;

FIG. 6 shows an image biological viewer in accordance with a preferred embodiment;

FIG. 7 shows a correlation plot biological viewer in accordance with a preferred embodiment;

FIG. 8 shows a trend plot biological viewer in accordance with a preferred embodiment;

FIG. 9 shows a biological response profile cluster tree;

FIG. 10 shows a cluster tree biological viewer in accordance with a preferred embodiment;

FIG. 11 shows the cluster tree biological viewer of FIG. 10 at a different magnification and scale;

FIG. 12 shows a flowchart of a dynamic biological menu generation algorithm in accordance with a preferred embodiment;

FIG. 13 shows a main search window in accordance with a preferred embodiment having an expanded criteria menu;

FIG. 14 shows the main search window of FIG. 13 after a search has been performed;

FIG. 15 shows the main search window of FIG. 13 with a pulled-down dynamic search condition menu;

FIG. 16 shows steps for computer-assisted analysis of biological response data in accordance with a preferred embodiment;

FIGS. 17-19 show examples of projections of selected biological data in accordance with a preferred embodiment;

FIGS. 20A and 20B show steps for finding the common parent node of two biological signal profiles on a hierarchical cluster tree in accordance with a preferred embodiment; and

FIG. 21 shows a conceptual diagram corresponding to steps for constructing a gene signal profile from a plurality of experimental profiles in accordance with a preferred embodiment.

FIG. 22 shows a user menu that allows for subtraction of biological signal profiles or combinations thereof from one another.

FIG. 23 shows a "resolved" profile that is the result of subtracting an Experiment profile of yeast cells harboring an impaired version of a gene, ERG11 and an Experiment profile of yeast cells treated with the drug clotrimazole.

FIG. 24 shows a biological viewer, which is displaying biological data in plot form, and a context-sensitive menu in accordance with one embodiment of the present invention.

FIG. 25 shows a biological viewer, which is displaying biological data in tabular form, and a context-sensitive menu in accordance with one embodiment of the present invention.

FIG. 26 shows a URL that has been accessed using a link in a context-sensitive menu, in accordance with one embodiment of the present invention.

FIGS. 27-29 show an experiment search tool wizard in accordance with one embodiment of the present invention.

FIGS. 30-32 show a gene search tool wizard in accordance with one embodiment of the present invention.

## 5. DETAILED DESCRIPTION

This section presents a detailed description of the invention and its applications. In particular, Section 5.1 generally describes the methods of the invention. Sections 5.2 and 5.3 describe, in detail, types of biological response signals which may be analyzed according to the methods of the present invention, as well as methods for obtaining such biological response signals. In particular, Section 5.2 describes methods of measuring cellular constituents and Section 5.3 describes various targeted methods of perturbing the biological state of a cell or organism.

The description is by way of several exemplary illustrations, in increasing detail and specificity, of the general methods of the invention. The examples are non-limiting, and related variants that will be apparent to one skilled in the art are intended to be encompassed by the appended claims. Following these examples are descriptions of embodiments of the data gathering steps that accompany the general methods.

### 5.1. OVERVIEW OF THE METHODS OF THE INVENTION

Preferred embodiments are described herein with respect to one example of a system yielding biological response signals, although it is to be appreciated that the scope of the preferred embodiments is not so limited and may be applied to any of a variety of experimental environments involving biological response signals. An expression array is a microarray adapted to generate light signals at each matrix site responsive to an amount of mRNA being expressed for a particular gene product at that site. Such systems are generally described in U.S. Pat. App. No. 09/179,569, "Methods for Using Co-regulated Genesets to Enhance Detection and Classification of Gene Expression Patterns," Attorney Docket No. 9301-031-999, filed October 27, 1998; U.S. Pat. App. No. 09/220,142, "Methods of Characterizing Drug Activities Using Consensus Profiles," Attorney Docket No. 9301-035-999 filed December 23, 1998; U.S. Pat. App. No. 09/220,274, "Methods for Robust Discrimination of Profiles," Attorney Docket No. 9301-036-999, filed December 23, 1998; and U.S. Pat. App. No. 09/220,275, "Methods of Clustering Analysis for Identification of Biological Pathways and Cluster Significance Assignment," Attorney

Docket No. 9301-039-999 filed December 23, 1999. The contents of each of the these applications is hereby incorporated by reference into the present application. Moreover, all publications cited herein are incorporated by reference in their entirety.

Microarrays are known in the art and consist of a surface to which probes that correspond in sequence to genes or gene products (*e.g.*, cDNAs, mRNAs, cRNAs, polypeptides, and fragments thereof), can be specifically hybridized or bound at a known position. The microarray is an array (*i.e.*, a matrix) in which each position represents a discrete binding site for a gene or gene product (*e.g.*, a DNA or protein), and in which binding sites are present for most or almost all of the genes in the organism's genome.

As disclosed *supra*, a perturbation includes but is not limited to the exposure of a biological sample to a drug candidate, the introduction of an exogenous gene into a biological sample, the deletion of a gene from the biological sample, or changes in the culture conditions of the biological sample. Responsive to a perturbation, a gene corresponding to a microarray site may, to varying degrees, be (a) upregulated, in which more mRNA corresponding to that gene may be present, (b) downregulated, in which less mRNA corresponding to that gene may be present, or (c) unchanged. The amount of upregulation or downregulation for a particular matrix location is made capable of machine measurement using known methods which cause photons of a first wavelength (*e.g.*, green) to be emitted for upregulated genes and photons of a second wavelength (*e.g.*, red) to be emitted for downregulated genes.

After perturbation and appropriate processing of the microarray, the photon emissions are scanned into numerical form, and an image of the entire microarray is stored in the form of an image representation such as a color JPEG format. The presence and degree of upregulation or downregulation of the gene at each microarray site represents, for the perturbation imposed on that site, the relevant output data for that experimental run or "scan."

FIG. 1 shows a biological response data network 100 for storage, retrieval, and analysis of biological response data in accordance with a preferred embodiment. FIG. 1 shows a data network 102 coupled to a scanning device 104, a database server 106, a computational server 107, an exemplary user computer 108, and a gateway computer 110. Gateway computer 110 is coupled to the Internet 112 which, in turn, is coupled to a remote scanning device 114 and a remote user computer 116. While a single instance of each of the above elements is disclosed in FIG. 1 for purposes of clarity of disclosure, it is to be appreciated that a typical implementation will generally include a plurality of user computers, data networks, scanning devices, computational servers, database servers, etc., in accordance with the preferred embodiments.

Data network 102 generally corresponds to a private local area network (LAN) such as an Ethernet, Token Ring, or FDDI (Fiber Distributed Data Interface) LAN, although the scope of the preferred embodiments is not so limited. Indeed, data network 102 may also comprise a wide area network (WAN) coupling LANs distributed across many corporate or university sites coupled by data bridges, routers, and switches as necessary. Protocols for coupling various sites corresponding to data network 102 may include X.25, SMDS (Switched Multimegabit Data Service), Frame Relay, ATM (Asynchronous Transfer Mode), or other data protocols as necessary. In general, the data network 102 should be capable of providing high-speed data communications between the database server 106 and other nodes in the network.

FIG. 2 shows a JPEG image 200 of an expression array scan produced by scanning device 104. While JPEG image 200 appears in black-and-white in FIG. 2 for ease of photocopy distribution of the present disclosure, it is actually a color image as known in the art, and represents the primary data generated from any gene/protein expression level measurement technology. As indicated in FIG. 1, scanning device 104 comprises a signal detector/transducer for scanning an expression array using methods known in the art to provide experimental data in the form of the color JPEG image 200. The color JPEG image 200 is transmitted to computational server 107 and database server 106 using the networking protocol appropriate for the data network 102. A significant amount of processing may be performed by the scanning device 104 or computational server 107 prior to or following transmission of the color JPEG image 200 to database server 106. The data resulting from this processing is generally a set of alphanumeric data identifying various characteristics of the expression array, such as quantitative signal intensity levels, expression ratios, p-values, the total number of usable sites on the expression array, and a large amount of other information known in the art. This alphanumeric data is sent in along with JPEG image 200 for storage in the database server 106. Remote scanning device 114 comprises similar hardware and software for performing tasks similar to scanning device 104, except that remote scanning device 114 is adapted to communicate with database server 106 over the Internet 112 using, for example, a TCP/IP protocol.

It is to be appreciated that scanning device 104 and the corresponding color JPEG image 200 represent only a single example of biological response signal data systems in accordance with a preferred embodiment, and that other systems for generating biological response signals are within the spirit and scope of the preferred embodiments. The analytical tools disclosed herein are broadly applicable to gene and protein expression data. For example, biological response signal data including gene expression level data generated from serial analysis of gene expression (SAGE, *supra*) (Velculescu *et al.*, 1995, Science,

270:484) and related technologies are within the scope of data suitable for analysis of the preferred embodiments. Other methods of generating biological response signals suitable for the preferred embodiments include, but are not limited to: traditional Northern and Southern blot analysis; antibody studies; chemiluminescence studies based on reporter genes such as lucerifase or green fluorescent protein; Lynx; READS (GeneLogic); and methods similar to those disclosed in U.S. Pat. No. 5,569,588 to Ashby et. al., "Methods for drug screening," the contents of which are hereby incorporated by reference into the present disclosure.

It is to be further appreciated that biological response signals that are suitable for analysis by the methods of the preferred embodiments are not limited to analog signals but can include binary event representations. For instance, in some embodiments, the biological response signal that is measured may be considered a "1" if a cellular constituent which comprises the signal is present in the biological sample and a "0" if it is not present. Thus, using a binary representation, the biological signal data considered by the methods of the preferred embodiments can be derived from expressed sequence tag (EST) approaches (Adams, *et al.*, 1992, Science, 252:1651), RNA blotting, ribonuclease protection and reverse-transcriptase-polymerase chain reaction analysis (Alwine *et al.*, 1977, Proc. Natl. Acad. Sci. U.S.A., 74:5350). As used herein the term "cellular constituent" includes individual genes, proteins, mRNA expressing a gene, and/or any other cellular component that is typically measured in a biological response experiment by those skilled in the art.

Data derived from methods that identify specific genes in a biological sample ("gene identification experiments") are also suitable for the methods of the present invention. In this context, the biological sample may be a cell line that has been incubated under controlled conditions. These controlled conditions may optionally include exposure of the cell line to a perturbation such as increasing concentrations of a pharmacological agent. Alternatively, the biological sample may be tissue obtained from a multicellular organism. Regardless of the type of biological sample utilized, there are a considerable number of experimental methods for identifying specific genes in such biological samples. Typically such experimental methods involve the extraction of the messenger RNA ("mRNA") from the cell line of interest. Complementary DNA ("cDNA") sequences are synthesized by reverse transcription of the extracted mRNA. Then, techniques that utilize specific oligonucleotide probes, which have been designed to selectively hybridize to particular DNA or gene sequences, are used according to the methods of Watson et al. See e.g. Watson et al., Recombinant DNA, chap. 7, W.H. Freemen, New York. For the purpose of obtaining a desirable form of input data for the methods of the present invention, data obtained by gene identification experiments may optionally be represented as binary events.

Thus if specific oligonucleotide probes identify a particular gene in a biological sample, such an event may be represented as a "1" whereas the inability to identify a gene of interest in a biological sample may be represented as a "0" or perhaps a "-1". More comprehensive illustrations of gene identification experiments are illustrated in the following paragraphs. One skilled in the art will readily appreciate that if the biological sample is first subjected to a perturbation, these gene identification experiments may be adapted to track differential expression of particular genes within the biological sample.

Often in gene identification experiments, the cDNA obtained from the extracted mRNA may be in the form of an arrayed cDNA library. An arrayed cDNA library is formed by placing the cDNA into vectors that are plated in a manner so that the progeny of individual vectors bearing the clone of one cDNA sequence can be separately identified. Replicas of such plates are then probed with a labeled DNA oligomer that has been selected to hybridize with the cDNA representing the gene of interest. As those colonies bearing the cDNA of interest are found and isolated, the corresponding cDNA inserts are harvested and sequenced by methods such as the Sanger dideoxy chain termination method (Sanger et al., 1977, "DNA sequencing with chain terminating inhibitors", Proc. Natl. Acad Sci. USA 74(12):5463-5467).

The DNA oligomer probes used in colony selection of cDNA libraries are synthesized to hybridize, preferably, only with the cDNA for the gene of interest. One manner of achieving this specificity is to start with the protein product of the gene of interest. If the sequence of a 5 to 10-mer peptide fragment from an active region of the protein product can be determined, corresponding 15 to 30-mer degenerate oligonucleotides that code for this peptide can be prepared. This collection of degenerate oligonucleotides will typically be sufficient to selectively identify the corresponding gene. Similarly, any experimental process that is capable of deriving a 15 to 30 long oligonucleotide gene subsequence can be used to create an oligonucleotide probe that is capable of selectively identifying the gene of interest.

Other types of gene identification experiments search for a known gene in a cDNA or genomic DNA prepared from a biological sample using single gene or single sequence probes that are complementary to unique subsequences of the known gene sequences. For example, the expression of a particular oncogene in biological sample can be determined by probing cDNA derived from the sample with a probe that is designed based on a subsequence of the oncogene's expressed sequence tag. Similarly the presence of a rare pathogen, such as the TB bacillus or the HIV, can be determined by probing gDNA with a hybridization probe specific to a gene of the pathogen. The heterozygous presence of a mutant allele in a phenotypically normal individual, or its homozygous presence in a fetus,

can be determined by probing with an allele specific probe complementary only to the mutant allele (See, e.g., Guo et al., 1994, Nucleic Acid Research, 22: 5456-65).

Another class of gene identification experiments include the method of sequencing by hybridization ("SBH"). SBH uses combinatorial probes that are not gene specific (Drmanac et al., 1993, Science 260:1649-52; U.S. Patent No. 5,202,231, Apr 13, 1993, to Drmanac et al). An exemplary implementation of SBH to identify a gene requires that a single cDNA clone be probed with all DNA oligomers of a given length, say, for example, all 6-mers. The complete set of all oligomers of a given length synthesized without any further criteria is known as a combinatorial probe library. From knowledge of all hybridization results for a combinatorial probe library, a partial DNA sequence for cDNA clones of interest can be reconstructed. Complete sequences are not determinable because, at least, repeated subsequences cannot be fully determined. SBH adapted to the classification of known genes is called oligomer sequence signatures ("OSS") (Lennon et al., 1991, Trends In Genetics 7(10):3 14-317). This technique classifies a single clone based on the pattern of probe hits against an entire combinatorial library, or a significant sub-library. It requires that the tissue sample library be arrayed into clones, each clone comprising only one pure sequence from the library. It cannot be applied to mixtures.

In contrast to the gene identification experiments outlined in the preceding paragraphs, another existing experimental technique, known as differential display, involves the "fingerprinting" of a mixture of expressed genes. The mixture of expressed genes may be, for example, a pooled cDNA library obtained from the total mRNA expressed by a biological sample. This fingerprinting seeks merely to distinguish two samples. No attempt is made to determine the quantitative, or even qualitative, expression of particular, determined genes (Liang et al., 1995, Current Opinions in Immunology 7:274-280; Liang et al., 1992, Science 257:967-71; Welsh et al., 1992, Nucleic Acid Res. 20:4965-70; McClelland et al., 1993, Exs 67:103-15; Lisitsyn, 1993, Science 259:946-50). Differential display uses the polymerase chain reaction ("PCR") to amplify DNA subsequences of various lengths, which are defined by being between the hybridization sites of arbitrarily selected primers. Ideally, the pattern of lengths observed is characteristic of the tissue from which the library was prepared. Typically, one primer used in differential display is oligo(dT) and the other is one or more arbitrary oligonucleotides designed to hybridize within a few hundred base pairs of the poly-dA tail of a cDNA in the library. Thereby, on electrophoretic separation, the amplified fragments of lengths up to a few hundred base pairs should generate bands characteristic and distinctive of the sample. Changes in tissue gene expression may be observed as changes in one or more bands. These and other gene identification experiments are more fully described in PCT Publication No. WO 97/15690,

"Method and Apparatus for Identifying, Classifying, or Quantifying DNA Sequences in a Sample Without Sequencing," published on May 1, 1997, the contents of which are hereby incorporated by reference into the present disclosure.

The methods outlined in the preceding paragraphs can collectively be termed biological response signal experiments. One skilled in the art will recognize that biological response signal experiments can be coupled based on a perturbation. For example, a pair of biological response signal experiments can be performed using a biological sample. One member of the pair of biological response signal experiments is run before the biological sample is subjected to a perturbation and the second member of the biological response signal experiments is performed after the sample has been subjected to a perturbation. After the pair of biological response signal experiments is run, each cellular constituent tracked by the pair of biological response signal experiments is compared. In one embodiment, this data is compared by representing cellular constituent that were present after introduction of the perturbation as a "1", representing cellular constituents whose presence was not altered by the perturbation in the coupled experiment as a "0", and representing cellular constituent that were present prior but not after the perturbation as a "-1". The reduced biological response signal data may then be analyzed in accordance with the preferred embodiments.

Database server 106 and computational server 107 typically comprise very high powered processors for processing large amounts of data, preferably arranged into a dual processor or multiprocessor systems. A suitable dual processor server may correspond, for example, to a Compaq DIGITAL™ Server 1200 equipped with two Pentium®-II processors, a Sun Enterprise™ 250 Server equipped with two UltraSPARC™-II processors, or a Compaq Alphaserver™ GS140 Server equipped with dual Alpha 21264 processors. A suitable multiprocessor server having four or more processors may correspond, for example, to a Compaq DIGITAL™ AlphaServer 8400 or a Sun Enterprise™ 10000 Server. Database server 106 will typically also comprise large, high performance storage media for data storage.

Database server 106 and computational server 107 each include an operating system such as UNIX or Windows® NT, along with hardware and software necessary to achieve a data communications interface with user computer 108, scanning device 104, remote scanning device 114, remote user computer 116, and generally any computer that is coupled to the Internet 112 using any of the communication protocols discussed *supra*. In a preferred embodiment, the database server 106, the computational server 107, the data network 102, and the gateway computer 110 are adapted to communicate with Internet 112 users using a TCP/IP protocol.

Database server 106 serves as the host site for a relational database management system for storing and retrieving biological data in accordance with a preferred embodiment. A database management system is a software program that typically operates on a database server or mainframe system to manage data, accept queries from users, and respond to those queries. A typical database management system is capable of: providing a way to structure data as records, tables, or objects; accept data input from operators and store that data for later retrieval; provide a query language for searching, sorting, reporting, and other decision support activities that help users correlate and make sense of the collected data; providing multi-user access to the data, along with security features that prevent some users from viewing and/or changing certain types of information; providing data integrity features that prevent more than one user from accessing and changing the same information simultaneously; and providing a data dictionary that describes the structure of the database, related files, and record information.

Most database management systems, such as that hosted by database server 106, are client/server based and operate over networks. In the embodiment of FIG. 1, the server is the database server 106, whereas the clients include user computers 108 and 116, as well as scanning devices 104 and 114. Database management systems include an engine that runs on a powerful server with a high-performance channel to the large data store. The database server 106 system accepts requests from the clients that may require sorting and extracting data. Once the database server 106 has processed the request, it returns the information to the client. The common language for accessing most database systems is SQL (Structured Query Language). In a preferred embodiment, database server 106 uses an Oracle database management system that operates responsive to SQL queries.

Although within the scope of the preferred embodiments, flat-file database systems are not recommended for use in biological response data network 100. Flat-file databases are generally applicable to simple data systems, since all the information can be stored in one file. Flat-file databases are generally inadequate for complex database applications such as that of biological response data network 100. Rather, relational database systems and/or object-oriented database systems are more appropriate for the biological response data network 100. A relational database management system in accordance with the preferred embodiments is a system that stores biological data in multiple tables. The tables can be related and combined in a number of ways to correlate and view the data. A typical database for a biological analysis system might contain hundreds of tables that can potentially produce thousands of relationships. A common element, such as a scan ID or a gene ID, may link information across the tables. A query for a particular scan, for example,

may pull the scan date from a first table, the involved genes from a second table, the perturbation types from a third table, and so on.

Object-oriented databases, which are also within the scope of the preferred embodiments, generally include the capabilities of relational databases but are capable of storing many different data types including images, audio, and video. Additionally, object oriented databases are adapted to store methods, which include properties and procedures that are associated with objects directly in the database. A variety of references are publicly available for further information on implementing relational and/or object oriented databases for enabling the implementation of the systems and methods disclosed herein; see, for example, Cassidy, *High Performance Oracle8 SQL Programming and Tuning,* Coriolis Group (March 1998), and Loney and Koch, *Oracle 8 : The Complete Reference (Oracle Series)*, Oracle Press (September 1997), the contents of which are hereby incorporated by reference into the present disclosure.

User computer 108 comprises a computing device or workstation capable of implementing biological response analysis software in accordance with a preferred embodiment. User computer 108 may correspond, for example, to a personal computer having a Pentium®-II processor and a Windows® NT Workstation operating system, to a Sun workstation having a SPARC processor and a UNIX operating system, or other similar systems. Although the scope of the preferred embodiments is not so limited, user computer 108 is generally not required to have computing power comparable to that of database server 106, although it is preferable for user computer 108 to have a high degree of graphics capability for displaying multiple graphical windows to the user. Remote user computer 116 is similar but is also adapted to communicate with database server 106 over the Internet 112 using, for example a TCP/IP protocol as disclosed *supra*. Although the scope of the preferred embodiments is not so limited, a typical biological response data network 100 will comprise a plurality of user computers 108 and a plurality of remote user computers 116 for each such database server 106. Thus, many users may simultaneously perform analysis on the biological response data stored in database server 106.

FIG. 3 shows a block diagram corresponding to three primary software elements of the biological response data network 100, in particular software corresponding to the scanning device 104, the database server 106, and the user computer 108. More specifically, FIG. 3 shows biological database software 302 corresponding to the database server 106, biological response analysis software 304 corresponding to the user computer 108, and scanning/preprocessing software 306 corresponding to scanning device 104. For simplicity and clarity of disclosure, the function of the computational server 107 is integrated into the database server 106 for the purposes of FIG. 3, although it is to be

understood that the scope of the preferred embodiments extends to systems that may separate their respective functions into different hardware systems. Importantly, it is to be appreciated that while the software elements of FIG. 3 are shown corresponding to separate computing devices, any or all or their functionality may be carried out in fewer devices, e.g. a single computer system, or in a far greater number of distributed devices having dedicated computers or even application specific integrated circuits (ASICs) for each specific function, without departing from the scope of the preferred embodiments.

FIG. 3 shows biological scanning/preprocessing software 306 as comprising a single block. Using methods known in the art, biological scanning/preprocessing software 306 performs the steps of (a) directing the physical scanning apparatus to read the scans into image files and/or other primary data, (b) processing part or all of the biological response data, and (c) transferring the biological response data including image files and/or other primary data to database server 106.

FIG. 3 shows biological database software 302 as comprising a communications interface module 308, a biological information processing module 310, a database 312, and a SQL query processing/response module 314. Communications interface module 308 coordinates data transfer between the database server 106 and the scanning device 104 for receiving biological response information. Communications interface module 308 also coordinates data transfer in the form of SQL database queries and responses between the database server 106 and the user computer 108. Biological information processing module 310 processes as necessary any biological information that was not already preprocessed by the biological scanning/preprocessing software 306 such that the requisite data is in appropriate form for storage in database 312 or distilled for presentation by biological response analysis software 304. For example, as described further *infra*, biological information processing module 310 may precompute correlation coefficients among all pairs of experiments being loaded into the database, or automatically group repeated experiments together based upon information in the database. In accordance with a preferred embodiment, database 312 is a set of tables and/or other data objects stored in accordance with the Oracle 8® database management system. Finally, SQL query processing and response module 314 handles SQL queries received from the user computer 108, searches the database 312, and provides responses for communication back to user computer 108. Depending on the query type, this may involve the invocation of further processing by biological information processing module 310.

FIG. 3 also shows biological response analysis software 304 in accordance with a preferred embodiment, which is installed on user computer 108 and a plurality of other user computers in biological response data network 100. As shown in FIG. 3, biological

response analysis software 304 comprises a plurality of functional modules including a user interface module 316, a resolving module 318, an SQL query module 320, and a communications module 322.

Advantageously, according to a preferred embodiment, biological response analysis software 304 is written in the Java programming language, thus allowing for platform independence such that it may be executed on any of a variety of user computers 108 and remote user computers 116 having different operating systems. As known in the art, Java is a programming language optimized for cross-platform, object-oriented, distributed, multithreaded computing, which is particularly advantageous in view of the functionality of biological response analysis software 304. Information on Java programming may be found, for example, in Ritchey, *Java!*, New Riders Publishing (1995), and Lemay, Perkins, and Morrison, *Teach Yourself Java in 21 Days: Professional Reference Edition*, Sams.net Publishing (1996), the contents of both of these references being hereby incorporated by reference into the present application.

User interface module 316 comprises software for driving a menu-driven, multi-window graphical interface for allowing the user to easily manipulate and analyze data in oner or more viewer windows. In a preferred embodiment, the user interface module 316 is adapted to provide the look and feel of an Internet browser interface, a Windows® 95 interface, or an X-Windows type interface. Resolving module 318 comprises software for coordinating data presentation among multiple windows, processing search parameters, performing statistical analysis, projecting biological datasets, and other routines within biological response analysis software 304 in accordance with the preferred embodiments. SQL query module 320 comprises software for formulating SQL queries compatible with the Oracle 8® based database 312 responsive to the data requirements of biological response analysis software 304. Communications interface module 322 comprises software for transmitting SQL queries to database server 106 containing the database 312 and for receiving responses therefrom.

It is to be appreciated that the specific programming structure of biological response analysis software 304 may very significantly, but in all cases is enabled by the present disclosure, it being understood that one skilled in the art will be readily capable of implementing the preferred embodiments given the present descriptions and the referenced works. It is to be further appreciated that, as necessary *infra* to clearly describe the preferred embodiments, hypothetical biological examples may be used instead of actual biological materials, *e.g.* "Gene X", "Gene Y", "Drug A", etc.

FIG. 4 shows a query composition and results display window 400 corresponding to biological response analysis software 304 in accordance with a preferred embodiment. In

the example of FIG. 4 the biological response analysis software 304 is adapted for analysis of gene or protein expression data, although the scope of the preferred embodiments is not so limited and may include software for analyzing any type of biological experiment configuration that produces machine readable signals. Query composition and results display window 400 comprises an experiments tab 402, a scans tab 404, a biosets tab 406, and a genes tab 408 which, although used for searching among different groupings of expression array data, present similar search interfaces to the user when selected.

Query composition and results display window 400 comprises, within the currently active experiments tab 402, scans tab 404, biosets tab 406, or genes tab 408, as the case may be, an alphanumeric data display area 409 for displaying the selected set of experiments, scans, biosets, or genes. Data is generally listed in column format, the columns being adjustable and scrollable. It is to be appreciated that the data area 409 of FIG. 4, while showing several fields including Chip Barcode, Hyb Name (i.e. the type of perturbation), Scan Date, Analysis Date, Mean Sig/Bkgd, Sample (G), and Sample (R), may contain many different sets of fields as determined by the contents of biological response database 318, and may be right-scrollable for showing many more fields.

Generally speaking, the scans tab 404 is used to search for primary expression data from individual hybridizations, *i.e.*, readings from individual experimental perturbations. The experiments tab 402 is used to search for combined sets of scans derived from analysis of repeated hybridizations. The genes tab 408 is used to search for genes contained in the biological response database 312, which may include any arrayed substances including controls, PCR products, genomic data, and the like. Finally, the biosets tab 406 is used to search for collections of experiments or genes. Basic searching is performed by (a) selecting a search criteria item from the search criteria menu 410, (b) selecting a search condition from the search condition menu 412, (c) selecting or entering search values into search value field 414, and (d) pressing the execute launch button 415. The experiments, scans, biosets, or genes in biological response database 312 that match the search criteria appear in the alphanumeric data display area 409.

In general, and as will be further described herein, in a preferred embodiment the user operation of biological response analysis software 304 involves a first step of selecting a single set or a plurality of sets of experiments, scans, or genes by a query process similar to the above basic search process. Following this step, a second step may be performed wherein selected data is viewed using one or more biological viewers. An exemplary set of basic biological viewers includes an expression image biological viewer, a signature plot biological viewer, a table biological viewer, and a statistics biological viewer. Following this step, a third step may be performed wherein a subset of data being displayed on one of

the biological viewers is selected and projected onto other active biological viewers for further user analysis, the subset being identified based on a search for datapoints meeting specific conditions and/or based on other identification procedures. Depending on nature of the analysis that is being performed, useful results may occur after any of the above steps. Generally speaking, the above steps will be iteratively performed by the user whose biological research, including the search for biological pathways, is enhanced through the use of the biological response analysis software 304.

Alternatively or in conjunction with the above steps, an additional step of comparing several experiments, scans, or genes may be performed using a biological comparison algorithm (*e.g.*, "ROAST") and biological viewers associated therewith. An exemplary set of biological viewers associated with the biological comparison algorithm (*e.g.*, ROAST) includes a correlation plot viewer. Finally, either alternatively or in conjunction with all of the above steps, a further additional step of forming defined biological sets may be performed in conjunction with the use of still other biological viewers. An exemplary set of biological viewers associated with the analysis of defined biological sets includes a trend biological viewer and a cluster tree or gene coregulation tree biological viewer.

Query composition and results display window 400 further comprises a plot launch button 416 that is functional when the experiments tab 402, scans tab 404, or genes tab 408 is active. In particular, when pressed by the user, the plot launch button 416 launches a signature plot biological viewer, a software routine that creates a separate display window showing a signature plot for the selected experiment or scan, or gene.

FIG. 5 shows a signature plot viewer 500 in accordance with a preferred embodiment. Signature plot viewer 500 appears in a separate window than the query composition and results display window 400 when launched. While signature plot viewer 500 is shown in FIG. 5 as plotting the base-10 logarithm of expression ratio versus the base-10 logarithm of intensity, many different scales may be used. At the selection of the user, gene labels may activated on the display for viewing one or more specific gene names. One such label 502 appears in FIG. 5 as an example. Further options are made available to the user for more optimal display of the signature plot viewer 500. As a nonlimiting example, the user is permitted to enter commands that highlight groups of genes (upregulated, downregulated, signature, controls, flagged spots, or a custom sets) in a particular color, for example, genes with a p-value less than 0.1 and a log(expression ration) greater than 0 could be highlighted in the color red. As a further nonlimiting example, the user is may enter commands that highlight all upregulated genes above, for example, 0.5, in the color red, and all downregulated genes below, for example, -1.0, in the color green for easier viewing.

With reference to FIG. 4, query composition and results display window 400 further comprises a table launch button 418 that is functional when the experiments tab 402, scans tab 404, or genes tab 408 is active. The table launch button 418 launches a table biological viewer, a software routine that creates a separate display window (not shown) showing alphanumeric data in spreadsheet format corresponding to the selected experiment, scan, or gene.

Query composition and results display window 400 further comprises an info launch button 422, which is functional when any of the experiments tab 402, scans tab 404, biosets tab 406, or genes tab 408 is active. The info launch button 422 invokes a statistics biological viewer (not shown) for viewing vital statistics for the selected experiment, scan, bioset, or gene. As an example, vital statistics for a selected experiment or scan may include quality information.

From the scans tab 404, an image launch button (not shown) may be executed by the user. The image launch button invokes an image biological viewer for viewing an image of the primary biological signal data, *e.g.*, the expression microarray as taken by scanning device 104, along with other data.

FIG. 6 shows an scan image 600 corresponding to an image viewer in accordance with a preferred embodiment. As shown in FIG. 6, a scan information panel 602 on the top of the display shows critical statistics for the scan including computed quality statistics, while the JPEG image 604 for the scan is shown at the left of the display. As known in the art, JPEG image 604 shows hundreds or thousands of spots, each corresponding to an individual gene. In accordance with a preferred embodiment, the image viewer includes the ability for the user to select an individual spot with a mouse button, which will then be shown in an enlarged spot image 606 as shown in FIG. 6. A spot information panel 608 displays critical statistics for that spot, including the p-value, fold-change, and other critical statistics known in the art.

Query composition and results display window 400 further comprises a biological comparison algorithm launch button (*e.g.*, "ROAST") 424 that is functional when the experiments tab 402, scans tab 404, or genes tab 408 is active. A biological comparison algorithm in accordance with a preferred embodiment allows the user to search and compare experiments, scans, or genes to each other in the biological response database 318.

Generally speaking, the biological comparison algorithm permits comparison of experiments to experiments, scans to scans, or genes to genes in a variety of advantageous manners. For example, with respect to experiments, the biological comparison algorithm enables the user to perform the steps of (a) searching the biological response database 318 for correlated response profiles, (b) obtaining a listing of similar response profiles

prioritized by degree of correlation (correlation coefficient, p-value, or other similarity metric), and (c) viewing a correlation plot of two such experiments.

Regarding step (a), the search for correlated experiments begins when the user identifies a "query" biological response profile. The query may correspond to any profile of particular interest to the user, *e.g.*, an experiment involving a cellular perturbation with a specific concentration of drug X. The user identifies the query by performing one or more searches using the query composition and results display window 400, selecting a query response profile, then pressing the a biological comparison algorithm ("ROAST") launch button 424.

Following the selection of the source set of experiments resulting from the query response profile, the user again presses the biological comparison algorithm launch button ("ROAST") 424. A wizard window resembling a search window then appears (not shown), allowing the user to select a target set of experiments. The target set of experiments may be as broad as the entire biological response database 312, or may be a specific target set, such as experiments involving cellular perturbations with various concentrations of drugs W, Y, and Z. Advantageously, in a biological comparison algorithm in accordance with a preferred embodiment, several such searches may be performed, the results being added to a shopping cart after each search, the final contents of the shopping cart being used as the target set.

Following the selection of the source sets and target sets, a correlation execution launch button (*e.g.*, "FINISH") is then pressed. The biological comparison algorithm then searches for profiles within the target set that are similar to the query profile by comparing similarity metrics among the pairs, then sorting the result list using the similarity metrics (*e.g.*, correlation coefficients) between experiments in the respective source and target sets. A NEXT button may optionally be pressed to "refine" the search. The user can select genes within an experiment profile or experiments within a gene profile for use in the computation of similarity metrics such as the correlation coefficient. If this step is skipped, all data points in the profiles will be used for the comparison.

Advantageously, in accordance with a preferred embodiment, the correlation coefficients between experiments are precomputed, that is, computed at a prior time nearer to a time when the experiments were first loaded from the scanning device 104 into the biological response database 312. The correlation coefficients are precomputed using methods known in the art and stored along with the experiment data in biological response database 318. While in the correlation coefficients are usually scalar numbers between 0.0 and 1.0, correlation data may alternatively comprise correlation matrices, p-values, or other similarity metrics in accordance with the preferred embodiments. Significant time savings

may be attained by precomputing the similarity metrics at a prior time, as there is no need for real time "on the fly" computation of the correlation coefficients by the user computer 108 after the correlation execution launch button ("FINISH") is pressed.

Regarding step (b) above for viewing an ordered list of correlations between the query profile and each of the target profiles, the search results in a preferred embodiment can be displayed in columns, usually ordered from highest to lowest correlation coefficient. Regarding step (c), the user may then select any target match listed therein, and then select a correlation plot launch button to spawn a correlation plot biological viewer.

FIG. 7 shows a correlation plot viewer 700 in accordance with a preferred embodiment. As with all viewers in a preferred embodiment, correlation plot viewer 700 appears in a separate window than the query composition and results display window 400 when launched, and also in a window separate from any other active biological viewer such as the signature plot viewer 500 *supra*. Each gene common to the two experiments is plotted on the correlation plot viewer 700 at a vertical axis location corresponding to its expression ratio or another measure of biological signal response in the first experiment (30 ug/ml CsA in the example of FIG. 7) and at a horizontal axis location corresponding to its expression ratio or another measure of biological signal response in the second experiment (1 ug/ml FK506 in the example of FIG. 7). While the correlation plot viewer 700 is shown in FIG. 7 as plotting the base-10 logarithm of expression ratios, many different scales or biological signal response measurements may be used. As is generally the case with all biological viewers disclosed herein, datapoint labels (*e.g.*, gene or experiment) may activated on the display for viewing one or more specific gene names, two such labels 702 and 704 appearing in FIG. 7. As with the signature plot viewer 500 of FIG. 5, several display options are available to the user for optimal viewing, such as color highlighting of genes that are significantly upregulated for both experiments.

Query composition and results display window 400 further comprises a bioset launch button 420, which is functional when the experiments tab 402 or genes tab 408 is active. When pressed by the user, bioset launch button 420 launches a software routine for creating, managing, and viewing biosets, that is, collections of experiments, genes, or other biological reagents. Biosets can be created for any of a variety of relationships among genes or experiments, such as members of a titration curve or other sets of experiments that may be of interest to the user. The biosets tab 420 contains data from gene and experiment clustering experiments, as well as other "ExperimentSets" or "GeneSets" that may be built by the user using intuitive menu-driven commands from within the experiments tab 402 or genes tab 408, respectively.

FIG. 8 shows a trend plot viewer 800 in accordance with the preferred embodiments. The trend plot biological viewer 800 is spawned from within the biosets tab 420 upon user selection of an experiment set and pressing of a trend plot launch button (not shown). As known in the art, trend plot viewer 800 comprises a set of titration curves for a set of genes, where the expression ratios are plotted for each gene versus a successively changing perturbation amount (Alpha factor concentration in FIG. 8). As with other biological viewers disclosed herein, datapoint labels may activated on the display for viewing one or more specific gene names, one such label 802 appearing in FIG. 8. As with the signature plot viewer 700 of FIG. 7, several display options are available to the user for optimal viewing, such as color highlighting of genes that are significantly upregulated or downregulated as the intensity of the perturbation factor increases.

FIG. 9 shows a biological response profile cluster tree 900 in accordance with the prior art. More specifically, FIG. 9 shows a gene coregulation tree, which corresponds to one type of biological response profile cluster tree. Using data obtained from multiple experiments on a set of genes, a gene coregulation tree may be constructed using methods known in the art for cluster analysis, grouping genes based upon commonality of gene responses to various biological perturbations. Generally speaking, response profiles that are highly similar to one another will be close together within the cluster tree, and significantly different profiles will be farther apart. Each gene eventually populates its own "leaf" on the end of the coregulation tree. Increasing distance between upstream nodes in common to two genes (profiles) is indicative of more significant dissimilarity between those two genes (profiles).

The prior art cluster tree 900 presents difficulties to computer users in analyzing its structure on a computer monitor. In particular, the lateral separation of branches in a single direction (the horizontal direction in FIG. 9) causes excessive crowding of lower branch nodes and a loss of perspective when zooming. For example, when zooming in on a lower level "outer" branch in a computer display of FIG. 9, the user quickly loses perspective of the big picture, *i.e.* where they are with respect to higher level nodes and to the origin or "root node" of the tree.

FIGS. 10 and 11 show computer display outputs of a cluster tree biological viewer in accordance with the preferred embodiments. The cluster tree biological viewer is launched from within the biosets tab 420 upon user selection of an ExperimentSet or GeneSet and the pressing of a tree plot launch button (not shown). In accordance with a preferred embodiment, a cluster tree is first computed using methods known in the art, and then adapted for display in a hyperbolic display format. By hyperbolic display format, it is meant that the gene coregulation tree appears on the computer screen as if it has been

mapped according to a hyperbolic mapping function. As shown in FIGS. 10 and 11, the specific hyperbolic mapping function used may be regulated by a user-selectable scale input 1002 and a user-selectable magnification input 1004.

Advantageously, the hyperbolically displayed gene coregulation trees shown in FIGS. 10 and 11 provide for convenient viewing of specific locations on the gene coregulation tree, without losing perspective of where the user is looking relative to the branches nearer to the "root" of the tree. As shown in FIG. 11, even at very close magnification near the specific genes ("leaves"), there is an immediate sense to the user as to the location of the center of the tree. As with other biological viewers disclosed herein, gene labels may activated on the display for viewing one or more specific gene names and several display options are available to the user for optimal viewing. For example, in a preferred embodiment, the user may hold the shift key down, and then click-and-drag using the mouse to rotate the tree about the "root" node. In a preferred embodiment, the user may invoke a "find common parent" algorithm to identify the parent node in common to all currently selected profiles (genes or experiments), one such algorithm being illustrated and explained by FIG. 20.

Advantageously, hyperbolic displays similar to those of FIGS. 10 and 11 may be utilized for analyzing expression array data using the steps of (a) generating a coregulation tree using expression array data, (b) displaying the coregulation tree in a hyperbolic display format, (c) manipulating the display of the coregulation tree to display a first gene or gene product located on a branch thereof, the first gene or gene product having a known function, (d) locating a second gene or gene product on the coregulation tree on the same branch as the first gene or gene product, and (e) assigning a function to the second gene or gene product using information related to the known function of the first gene or gene product and information related to a positional relationship or distance metric between the second gene or gene product and the first gene or gene product on the branch of the coregulation tree. Assignment of function includes, but is not limited to, the identification of new drug targets by starting with known drug targets on the tree. Given one or more known successful drug targets, this technique allows the identification of new prospective drug targets involved in the same cellular pathway or process based on their positional relationship with known targets on the cluster tree (or distance metric between them).

In another preferred embodiment, hyperbolic displays similar to those of FIGS. 10 and 11 may be utilized for analyzing expression array data using the steps of (a) generating an experiment profile cluster tree using the expression array data, (b) displaying the experiment profile cluster tree in a hyperbolic display format, (c) manipulating the display of the experiment profile cluster tree to display a first experiment located on a branch

thereof, the first experiment corresponding to a known function of a perturbation assayed therein, (d) locating a second experiment on the experiment profile cluster tree on the same branch as the first experiment, and (e) assigning a function to a biological perturbation assayed in the second experiment using information related to the known function of the perturbation assayed in the first experiment and information related to a positional relationship or distance metric between the second experiment and the first experiment on the branch of the experiment profile cluster tree. Assignment of function includes, but is not limited to, the identification of new drug targets by starting with known drug targets on the tree. Given one or more known successful drugs or drug target deletion profiles, this technique allows the identification of similar profiles that represent new prospective drugs or drug targets affecting or involved in the same cellular pathway or process.

FIG. 12 shows a flowchart of a dynamic biological menu generation algorithm 1200 in accordance with a preferred embodiment. FIG. 13 shows the query composition and results display window 400 with the criteria menu 410 expanded to illustrate the steps 1200 of FIG. 12. As shown in FIG. 13, the criteria menu 410 comprises a primary menu 1302 and a series of submenus, some of which are shown as submenus 1304, 1306, 1308, and 1310. The user activates the primary menu 1302 by clicking in the relevant space. The primary menu 1302, as well as the submenus 1304, 1306, 1308, and 1310 each comprise one or more of the following: (a) field labels, representing final biological field choices for the criteria menu 410, such as Chip Barcode, Hyb Date, Hyb Name, Hyb Type, Scan Date, Control, #/Scans in Group, and generally all items in that menu except for those labeled with a marker (➤); and biological subcategory labels, characterized by a marker (➤), which do not represent field choices but rather are indicative that further biological subcategory items to be displayed are available and queried upon.

In accordance with a preferred embodiment, the primary menu items and submenu items including all field labels, biological subcategory labels, and relationships among them are not "hard-coded" into the biological response analysis software 304. Rather, they are obtained from the database at program initiation for allowing increased flexibility and customizability. Thus, at step 1202, the biological response analysis software 304 is initiated ("started up") by the user. At step 1204, biological response analysis software 304 retrieves dynamic menu information from the biological response database 312, the dynamic menu information including the primary menu items and submenu items including all field labels, biological subcategory labels, etc., needed for menuing. By dynamic menu information, it is meant that this menu information may be changed by simply changing the contents of the biological response database 312 without the need for changing the "hard-coding" of the biological response analysis software 304.

At step 1206 the user selects a primary menu item, which may be either a biological field label or a biological subcategory label. At step 1208 it is determined whether the user has selected a biological subcategory label. If not, then the user has selected a biological field label, and the menu selection process is complete, which is then followed by step 1215. If a biological subcategory label was chosen at step 1208, the dynamic menu information that was retrieved at program start-up is used at step 1210 to construct and display the subcategory items on a submenu such at submenu 1304 in FIG. 13. At step 1212, the user selects an item from the submenu and the process is repeated until a biological field label is chosen, in which case the menu selection process is complete, which is then followed by step 1215.

At step 1215, the valid operators for the selected menu item are displayed to the user. At step 1216, the user is provided with the appropriate interface for selection or entry of a value for the selected menu item for search. At step 1217, the user adds more search criteria if necessary by repeating the above steps as appropriate. At step 1218, a database query is constructed using the user-entered data and data relationships stored in the database for transmission to the biological database software 302. In the example of FIG. 13, the user selects biological subcategory labels "Sample(s)," "Strain," and "Full Genotype" from the successive menus and submenus 1302, 1304 and 1306, respectively, and is in the process of finally selecting a biological field label "Gene Name" in submenu 1308.

Advantageously, the dynamic biological menu generation algorithm shown in FIGS. 12 and 13 allows for an enhanced degree of flexibility and customization in the implementation of biological response analysis software 304, as it no longer needs to be programmed directly with the submenu data shown at submenus 1304, 1306, and 1308 of FIG. 13. Rather, the they are obtained from the database at program initiation at step 1204. As a result, one or more menuing systems of the biological response analysis software 304, such as the criteria menu 410, is fully changeable by loading the appropriate data into the biological response database 312, and programming or updating of the biological response analysis software 304 is not required. From a practical implementation perspective, this is advantageous because the modification of the biological response database 312 is easier to implement than widespread updating of the biological response analysis software 304. Moreover, because different databases 312 (as discussed *supra*, the database 312 may be only one of many such databases) may correspond to different research entities, each research entity having its own specific menuing needs, flexibility is achieved because the separate entities may be provided with the same biological response software 304, and their differing menuing needs may be met simply through a custom programming of their respective databases 312. In a biological response data network 100 according to the

preferred embodiments, when a change is desired in biological response analysis software 304 menuing systems, or when the nature of the experiments change for providing different biological response results, or when a specific site or user requires specific needs, changes may be implemented quickly and efficiently through the relatively simple process of changing the biological response database 312.

FIG. 14 shows a exemplary main search menu display screen after a search has been performed, wherein the user has selected "Gene Name" in the experiments tab 402 from the dynamic biological menu generation algorithm of FIG. 12 for the criteria menu 410, has selected the condition "begins with" from the condition menu 412, and has entered the character string "rnr" into the value field 410. As shown in FIG. 14, only 3 of the 1,046 records contained in the biological response database 312 of that example contained that entry.

FIG. 15 shows a pull-down menu corresponding to the condition menu 412 under the experiments tab 402, showing generally the types of conditions that may be imposed on the search criteria. In the example of FIG. 15, the user has selected the condition "equal to," and has entered the search value "PAC 2." As shown, only a single experiment in the biological response database 312 is found to involve a perturbation of the PAC2 gene. It is to be appreciated that while the condition menu 412 does not contain subcategory items in the example of FIG. 15, such configuration is within the scope of the preferred embodiments. The condition menu 412 can also dynamically change to a selection interface if a criteria or condition selected is appropriate to a particular value interface. For example, when the "Hyb Type" criteria is selected, which has a small number of possible distinct values, a selector is displayed to allow easy selection from among the possibilities.

FIG. 16 shows steps for computer-assisted analysis of biological response data in accordance with a preferred embodiment, the steps being used in conjunction with the biological response analysis software 304. It has been found that it is desirable not only to provide computer software for analyzing biological data using the separated biological viewers disclosed *supra*, but also to integrate these biological viewers by allowing the projection of selected datasets onto these biological viewers. In one preferred embodiment, the dataset to be projected is selected according to search methods disclosed *supra* and projected onto all currently active biological viewers, using appropriate highlighting such as color highlighting. In another preferred embodiment, a source dataset is selected from a source viewer, *i.e.*, a first currently active biological viewer, and projected onto one or more destination viewers, *i.e.*, one or more of the other currently active biological viewers. The source dataset is projected onto the destination biological viewers through the highlighting of destination data points that correspond to the source dataset, making these data points

stand out for clear recognition by the user, thus enhancing the ability to recognize relationships, trends, patterns, etc. in the biological response database. This also allows the user to identify a meaningful response using a viewer well-suited to a particular purpose, then see the same response in other viewers to support or refute the discovery.

FIG. 17 shows a projection of selected biological data in accordance with a preferred embodiment, wherein a source dataset 1700 selected from a source viewer 1702 is projected onto a destination viewer 1704. The steps of FIG. 16 will be described with reference to the projection of FIG. 17, it being understood that the scope of the preferred embodiments is not so limited. At steps 1602 and 1604, the user selects and displays experiment, scan, bioset, gene, or other biological response datapoints taken from biological response database 312 in accordance with the search and display algorithms described *supra* in the present disclosure, and displays them in "N" biological data viewers simultaneously on the display screen of user computer 108. In FIG. 17, for example, the user has chosen a particular scan and displayed it using the signature plot biological viewer in window 1702, and has also chosen a particular scan (which may be the same scan or another scan having some genes in common) and displayed its data using the expression image biological viewer in window 1704. Although the number of active biological viewers "N" is only 2 in the example of FIG. 17 for simplicity and clarity of disclosure, "N" may be quite large in practice as windows may overlap, and is limited only by the size of the display screen and the computational resources available in user computer 108.

At step 1606, the user selects the source dataset 1700 from a source viewer using a graphical and/or query-based selection process. In the example of FIG. 17, the source dataset 1700 is circled using a lasso technique, wherein the user clicks the left mouse button while drawing a polygon around datapoints displayed on the plot. Accordingly, the source dataset comprises the set of genes corresponding to the circled locations on the plot. It is to be appreciated that many other graphical selection processes may be used in accordance with the preferred embodiments, *e.g.* multiple individual mouse clicks, circling with pen computing device pointers, etc. Also, query-based selection processes may be performed, wherein a database query is formulated using the "find" command from the edit menu.

At step 1608, the user executes a project selection command by pressing a project selection launch button or selecting a similar menu item. Responsive to the project selection command, biological response analysis software 304 causes the genes in the selected dataset to be highlighted in the destination viewer 1704, as shown in FIG. 17. The user then views the highlighted data at step 1610. It is to be appreciated that the arrow between windows in FIG. 17 is included only for clarity of disclosure, and does not actually show up on the display screen in a preferred embodiment. The example of FIG. 17 shows

highlighting of the projected dataset by the placement of arrows to the projected genes, which may be suitable for a binary black and white display environment (or, more particularly, a binary black and white photocopy environment as with the present disclosure). Preferably, however, the projected data points are highlighted using a different screen contrast, brightness, color differentiation scheme colors, or other marking methods suitable for drawing the attention of the user to the projected dataset.

In accordance with a preferred embodiment, the user is permitted to disable projections for any biological viewer window by selecting a "disable projections" option using a launch button or menu command made available in each biological viewer. When "disable projections" is activated, there will be no highlighting of data on that biological viewer during projections made from other biological viewers. The "disable projections" option is preferably made available on an individual window basis, and disabling projections on one biological viewer does not affect projection operations onto other biological viewers.

FIG. 18 shows a projection of selected biological data in accordance with a preferred embodiment. In FIG. 18, a source dataset 1800 from a first signature plot biological viewer 1802 is projected onto a second signature plot biological viewer 1804 and onto a third signature plot biological viewer 1806. The source dataset comprises the hypothetical genes A, B, C, D, E, and F that were circled using a lasso technique as described *supra*. In the hypothetical example of FIG. 18, the Biological response analysis software 304 may permit the user to more readily observe certain behaviors regarding genes A-F and drugs X, Y, and Z. In particular, the Biological response analysis software 304 may permit the user to quickly observe that the genes A-F, which are significantly upregulated by the drug X, are not all upregulated by drug Y, and indeed only gene A, gene B, and perhaps gene C are substantially upregulated. The user may also quickly observe that genes A, B, and C are substantially downregulated by drug Z and none are substantially upregulated.

FIG. 19 shows projection of selected biological data in accordance with a preferred embodiment. In FIG. 19, a source dataset 1900 from a signature plot biological viewer 1902 is projected onto a correlation plot biological viewer 1904 and onto a trend plot biological viewer 1906. Prior to projection of the source dataset 1900, the user has created the signature plot in biological viewer 1902 using the plot launch button 416 on the experiments tab 402 as described *supra*, and has also created the trend plot in biological viewer 1904 from the biosets viewer as described *supra*. The user may also make observations regarding the genes A-F and drugs X-Z from these dataset projections. It is to be appreciated there are many combinations of dataset projections that are possible in accordance with the preferred embodiments using the many biological viewers disclosed

*supra*. As a nonlimiting example, the source datasets may be taken from a gene coregulation tree biological viewer disclosed *supra* and projected onto any of the destination viewer of FIGS. 17, 18 or 19, or the source datasets may be taken from any of the destination viewer of FIGS. 17, 18 or 19 and projected onto the gene coregulation tree biological viewer.

With reference to FIG. 21, in accordance with the preferred embodiments, a cellular signal profile may be constructed from multiple experimental signal profiles rather than just a single experimental signal profile. As used herein, when multiple experimental signal profiles are combined into a single experimental signal profile, each experimental signal profile that was combined is referred to as a constitutive experimental profile. By combining constitutive experimental profiles into a single cellular signal profile, the methods of the present invention, including the ability to group particular cellular constituents (such as gene expression levels) and the ability to compare and visualize response data, have been extended so that the same methods can be used to analyze multiple experimental signal profiles simultaneously.

As an alternative to combining all the data found in constitutive experimental profiles, the response of a single gene can be collected from each of the constitutive experimental profiles to form a "gene signal profile". Thus the gene signal profile represents the response of a particular gene in several constitutive experimental signal profiles. The individual responses of the particular gene in each of the constitutive experimental signal profiles is not averaged together to form the gene signal profile. Rather the individual responses are preserved in a multidimensional data structure.

The extension of the methods of the present invention to simultaneous analysis of gene response across multiple experiments has substantial advantages over prior art. For example, the expression level of a particular gene, or a family of particular genes, in response to a plurality of biological perturbations, such as increasing exposure to a pharmacological agent, can easily be determined using the methods of the present invention. In another example, the expression level of particular genes and/or any other types of measurable experimental biological response data can be correlated across multiple diverse experiments in order to identify genes or signals that are coregulated with respect to the various perturbations used in the multitude of experimental signal profiles analyzed. Alternatively, because the plurality of experimental signal profile experiments is capable of establishing a "baseline" expression level and/or amount for each cellular constituent, simultaneous analysis of multiple experimental signal profiles allows for the identification of particular experimental signal profiles in which the response of particular gene or group of genes is unique. These methods further provide an excellent method for validating the

biological response of a pharmacological agent or test agent. By comparing the exposure of aliquots of a nominal biological sample, such as a particular cell line, in which each aliquot has been exposed to a reference compounds or the test compound, one can determine whether the test compound affects the same class of cellular constitutions as the reference compounds. Further, one can use the methods of the present invention to precisely define the relationship between the test compound and the reference compound over a multitude of perturbations including genetic manipulations of the biological sample.

To apply the methods of the present invention to multiple experiment signal profiles, it is necessary that the individual experiment profiles used for the construction of the gene signal profile be constructed such that, taken together, they yield one or more biological signal measurements for each of the genes whose profiles are being created. The experiments must also be normalizable. That is, the experiments must all yield data with common units, or data that is convertible to common units. For example, experiments based upon sequence tag based gene expression methods measure relative or absolute abundance of each gene transcript. Other experiments may measure gene or protein expression and/or activity levels and alternatively express these measurements as percent of transcript pool, copies per cell, hybridization intensity on Western blots or single-channel microarrays and related forms of measurement depending upon the nature of the experiment. Regardless of the form taken, all measurements must have the same units (dimension) and be directly applicable to the construction of composite gene signal profiles.

Dimensionless experiments, such as ratio-based measurements based upon multi-flour microarray experiments (Shalon *et. al.*, 1996, "A DNA Microarray System for Analyzing Complex Samples Using Two-Color Flourescent Probe Hybridization," Genome Research 6:639-645) can also be adapted for use according the methods of the present embodiment by insuring that each experiment included in the analysis has one or more measurements of reference cellular constituents (expressed as a ratio) relative to a "baseline" ratio. The "baseline" may be established by measuring one or more reference cellular constituents in all ratio-based experiments included in the analysis or it may be compared to a condition that is itself a common baseline or normalizable to a common baseline within the database of experiment profiles.

For example, with reference to FIG. 21, experiment signal profiles 1, 2, 3 and 4 are all ratio-based gene expression measurements. With data corresponding to...

Experiment Profile 1: GeneA Signal, GeneB Signal
Experiment Profile 2: GeneA Signal, GeneB Signal
Experiment Profile 3: GeneB Signal
Experiment Profile 4: GeneA Signal, Gene B Signal

...one can construct the following gene signal profiles:

GeneA: Experiment1 Signal, Experiment2 Signal, Experiment4 Signal
GeneB: Experiment1 Signal, Experiment2 Signal, Experiment3 Signal, Experiment4 Signal

This approach can be used for example to identify the experiments in which a particular gene or collection of genes is perturbed or affected.

The approach illustrated in this section has been used to identify experiments in which a particular gene or collection of genes is perturbed or affected. In the example of FIG. 5, a gene signal profile for a yeast gene RNR2 has been constructed using the RNR2 component of 863 biological signal profiles for experiments involving yeast gene expression monitoring of samples derived from cells with a variety of drug treatments or genetic perturbations. The profile exposes that RNR2 transcript levels are elevated with increasing concentration of the drug methotrexate (labeled MTX on plot). In that example, all experiments chosen to construct the RNR2 signal profile have a common baseline: yeast "wild type" untreated cells.

With reference to Fig. 22 and 23, in accordance with the preferred embodiments, a "Resolve" feature is provided that allows subtraction of biological signal profiles or combinations thereof from one another. The resolve feature can be used in many circumstances to validate drug targets. As shown in Fig. 22 and Fig. 23, exemplary profiles such as an Experiment profile based on yeast cells harboring an impaired version of a gene, ERG11, which is the target of the antifungal drug clotrimazole (ERG11; at left on Fig. 23), along with an Experiment profile of yeast cells treated with the drug clotrimazole (tet; at right on Fig. 23) can serve as input to the "Resolve" feature. The "resolved" profile, showing the differences between the two profiles (clotrimazole treated cell profile minus tet-ERG11 cell profile) is shown at the bottom of Fig. 23. The only significant outlier is the ERG11 gene, because it was turned off in the tet-ERG11 experiment, but remains active in the drug treated cell. This is indicative of a very precise drug that closely mimics the cellular effects of the deletion of its target. As this example illustrates, the "resolve" feature represents an method for validating the effects of drugs or for validating drug targets. While this illustration demonstrates the subtraction of one Experiment profile from another, one skilled in the art will appreciate that combinations of profiles may be subtracted from a single profile or from yet other combinations of profiles.

### 5.1.1. CONTEXT-SENSITIVE MENUS

Referring to Figure 24, another aspect of the present invention is disclosed. When a user selects a datapoint in a biological viewer, such as biological viewer 2000, a context-sensitive menu is provided. Similarly, when a biological viewer is displaying data in row

and column format, such as the format illustrated in Fig. 4, the context-sensitive menu may be activated by selecting a row and/or column of data. In one embodiment of the present invention, a datapoint or row of data is selected by "right-clicking" on the data element. For example, in Figure 24, menu 2402 is obtained by right-clicking on a spot in panel 2406.

The options provided in the context-sensitive menu are dependent on the object type that was selected by the user. For example, if the user selects a datapoint that represents a particular gene, the context-sensitive menu will provide the user with various options that relate to the study of genes. Other object types include, but are not limited to, experiments, profiles, proteins, and drugs. In one embodiment of the present invention, the options that are provided in a context-sensitive menu can be classified as (i) an analysis option ("command") or (ii) a link. In general, a different set of options and links are associated with each object type that is tracked in the present invention. Commands are described in further detail in section 5.1.1.1 and links are described in further detail in section 5.1.1.2.

### 5.1.1.1. COMMANDS IN CONTEXT-SENSITIVE MENUS

Analysis options ("commands") in the context-sensitive menus of the present invention represent any type of functionality that may be used to process a biological data object. For example, when the object is an "experiment", the context-sensitive menu associated with the data object class "experiment" may include a "plot" command. When the user selects the plot command, a plot is generated using the data in the selected experiment. In another non-limiting example, a gene object class may be associated with a context-sensitive menu that includes commands such as (1) a command to generate a plot of a selected gene's behavior across all experiments, (2) a command to translate a selected gene into a protein sequence, (3) a command to initiate a ROAST session using the selected gene, and/or, (4) a command to perform a BLAST search using the selected gene as the query.

Commands may include one or more subcommands. Each subcommand may, in turn, have any number of layers of subcommands. The context-sensitive menus of the present invention display commands and subcommands in a hierarchical manner. For example, in one embodiment, the commands are displayed in a single pane when the user right clicks on a datapoint or row. Context-sensitive menus of the present invention list all commands and links that are associated with the object class of the selected data element selected by the user.

The user scrolls through context-sensitive menus of commands and links. In one embodiment, only one of the commands or links is highlighted at any given point in time.

Furthermore, in such embodiments, when a command having one or more subcommands is highlighted, a submenu that includes the subcommands is displayed. The user may select any of the subcommands in the submenu or continue scrolling in the parent menu. The submenu is only displayed while the parent command in the parent menu is highlighted. There is no limit on the number of layers a command may have. That is, a command may have two layers and the commands may be termed commands (layer 1) and subcommands (layer 2). However, commands of the present invention could have three layers, four layers, or ten or more layers. Each time a command in an nth layer is highlighted, subcommands in the nth+1 layer that correspond to the nth command are presented in a submenu. Thus, it is possible, for example, that a parent menu, representing layer 1, a submenu representing a corresponding layer 2 subcommand, and a submenu representing a corresponding layer 3 command are simultaneously present in a biological viewer such as biological viewer 2400.

#### 5.1.1.2. LINKS IN CONTEXT-SENSITIVE MENUS

Links in context-sensitive menu may be used to connect to relevant biological resources that are related to the selected data element. In one embodiment of the present invention, links have a Uniform Resource Locator (URL) format. Advantageously, the links of the present invention may include substitution variables. As an example, consider a link that includes a <VALUE>primary_gene_name</VALUE> substitution variable. When a user clicks gene "Rec_A" in a plot, the <VALUE>primary_gene_name</VALUE> substitution variable in the link will be replaced with "Rec_A". Therefore, the URL, which likely designates a database, will link to a record that includes Rec_A. One of skill in the art will appreciate that substitution variables can be used to dynamically assign a link to a particular gene or protein of interest in a database such as Entrez, Medline, or NCBI.

Substitution variables, which are also known as tags, may include structured query language (SQL) expressions. Such expressions are particularly useful for creating highly customized links that are precisely tailored to provide information about the biological data object. As an illustration, consider the data in biological viewer 2502. Further shown in Figure 25 is context-sensitive menu 2504. In accordance with one embodiment of the present invention, a divider line separates command and link menu options in context-sensitive menu 2504. A row of biological data (2506) is highlighted in Figure 25. In column 2508 of row 2506, two different gene names are listed, SSA1 and YG100. A link could be added to context-sensitive menu 2504 that queries the Entrez database for any information related to the gene SSA1 by including an SQL expression within a tag used in a link. Such a link must satisfy two requirements (i) it must have the proper URL statements for querying the Entrez database and (ii) it must include a substitution tag that can be

replaced by the term SSA1. In one embodiment of the present invention, the latter requirement is supported by allowing SQL substitution tags in a label. In one example that is in accordance with the present invention, the SQL tag has the format <SQL>sql_text</SQL>, where sql_text is:

<SQL>SELECT primary_gene_name FROM a_gene WHERE gene_id=<VALUE>gene_id</VALUE></SQL>.

When a label includes such a SQL tag, biological analysis software 304 substitutes the value 9956 for "gene_id" and the primary_gene_name "SSA1" is returned. Therefore, the entire SQL tag is replaced with the name "SSA1" thereby satisfying requirement (ii) from above. Advantageously, SQL tags allow for complex useful expressions. For example, an SQL tag can be used in conjunction with a <REPEAT> tag to obtain all gene names in column 2508 of row 2506. By delimiting each of returned gene with a boolean OR operator, the Entrez database can be simultaneously searched for information relating to either the gene name SSA1 or YG100. In one embodiment of the present invention, the combination of a REPEAT tag and a <SQL> tag will have the format:

<REPEAT>+OR+<SQL>SELECT gene_name FROM a_gene_name WHERE gene_id=<VALUE>gene_id</VALUE></SQL></REPEAT>.

When the expression above is used to parse row 2506, the expression will return +OR+SSA1+OR+YG100. In addition to REPEAT tags and SQL tags, one embodiment of the present invention has VALUE tags. Value tags are replaced by biological analysis software 304 (FIG. 3) with the value of the column name within a biological data element that was selected. To use row 2506 as an illustration, the value in column 2510 could be included in a label by using the expression <VALUE>systematic_name</VALUE>. In this example, when a user selects row 2506, the value tag will be replaced by the value YAL005C.

Now that illustrative tags of the present invention have been described, more detailed examples of illustrative tags in accordance with the present invention may be described. In a first example, a context sensitive menu may include a link that will search the Entrez database for a particular gene. Now consider the case in which a biological viewer is displaying expression data for the yeast gene RTA1. When the user selects the gene RTA1 in the biological viewer to activate the context sensitive menu, one of the link

options may be the option to search Entrez for RTA1. When the user selects this menu option, biological analysis software 304 generates the URL:

http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=p&form=4&term=
(Saccharomyces+OR+yeast)+AND+(YGR213C+OR+RTA1)

using the link:

http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=p&form=4&term=
(<SQL>SELECT species_name FROM o_species WHERE species_id =
<VALUE>species_id</VALUE></SQL>)+AND+(<VALUE>
systematic_name</VALUE><REPEAT>+OR+<SQL>SELECT gene_name
FROM a_gene_name WHERE gene_id=<VALUE>gene_id</VALUE>
</SQL></REPEAT>).

In a second example, a link to the Proteome YPD having the URL expression:

http://customer.proteome.com/YPD/YGR213C.html

may be dynamically set up as the link:

http://customer.proteome.com/YPD/<VALUE>systematic_name</VALUE>.html

by use of the VALUE tag. In a third example, a link to the Stanford Saccharomyces Genome Database (SGD) site having the URL expression:

http://genome-www.stanford.edu/cgi-bin/dbrun/SacchDB?find+Locus+%
22YGR213C%22

may be dynamically set up as the link:

http://genome-www.stanford.edu/cgi-bin/dbrun/SacchDB?find+Locus+%
22<VALUE>systematic_name</VALUE>%22.

The links of the present invention may be nested. In one embodiment, URL that corresponds to a nested link is built by biological analysis software 304 (Fig. 3) by

resolving the mostly deeply nested tags first. This provides additional control over the context of REPEAT, SQL, and VALUE tags. In another embodiment, the breadth of SQL statements used in a SQL tag are restricted so that it is not possible to inadvertently overwrite or delete information in biological response database 312.

Now that detailed examples of this aspect of the invention have been disclosed, several advantages of this aspect of the invention will be appreciated. Using a context-sensitive menu, the user may rapidly access commands and links that are relevant to a selected biological data element. Additionally, context-sensitive menus may be used to provide all possible options to the user with respect to any given biological data element in one convenient location. Thus, the user advantageously does not have to "hunt" for commands of interest on several context insensitive menus. Further, the use of context-sensitive menus saves the user time because there is no need to enter information, such as the name of a gene of interest, into query forms. Commands and links in context-sensitive menu automatically know which biological data element the user is interested in. Further, by using substitution tags, including SQL tags, links can be customized for user-specific or site-specific applications.

The utility of links may be appreciated by referring to FIG. 25. When a user selects row 2506, context-sensitive menu 2504 is displayed. When the user selects the link to MEDLINE, an instance of a web browser 2600 (FIG. 26) is launched on user computer 108 and citations that include the gene SSA1 are displayed.

### 5.1.1.3. UPDATING CONTEXT-SENSITIVE MENUS

An advantage of the commands and links in the present invention is that they can be highly customized. In one embodiment, the commands and links of context-sensitive menus are stored in a flat file, database or a table that is accessible to biological analysis software 304 and/or biological analysis software 302. In another embodiment, the commands and links of context-sensitive menus are stored in machine readable form on server 106. As described *infra*, each context-sensitive menu is determined by a biological data type such as "gene," "experiment," *etc.* Thus, in one embodiment in accordance with the present invention, the links and commands of each biological data type are stored in separate files or tables on database server 106 and/or user computer 108. Commands and links may be added, edited, or deleted by editing the files or tables. A distinct advantage of one embodiment of the present invention is that context-sensitive menus may be updated without recompiling, updating, or altering biological analysis software 304 and/or biological analysis software 302.

### 5.1.2. SEARCH TOOL WIZARD

As described in detail *supra*, the present invention provides various biological comparison algorithms ("searches") that allow a user to search and compare experiments, scans, or genes to each other in the biological response database 318. Such comparisons may be initiated using an options such as button 424 ("ROAST," FIG. 4). An advantage of the present invention is that commands of section 5.1.1.1 may also be used to initiate such a search. Accordingly, when a biological data element, such as a single gene or experiment, is selected, the biological data element will be the "query." Further, the present invention contemplates the use of prompted inputs, hereinafter termed wizards, to facilitate the initiation of such searches. Wizards may be classified into at least two different categories: (i) experiment wizards, which are described in section 5.1.2.1, and (ii) gene wizards, which are described in section 5.1.2.2. Experiment wizards and the gene wizards are described in sections 5.1.2.1. and 5.1.2.2. using specific detailed embodiments. It will be appreciated, however, that any number of features may be added or deleted from the embodiments described in these sections and that all such embodiments are within the scope of the present invention. Furthermore, although the wizards present below are presented in an ordered succession of screens, it will be appreciated that the present invention is not limited to the exact ordering of the screens described below or, indeed, the total number of screens used in a particular wizard.

#### 5.1.2.1 EXPERIMENT SEARCH TOOL WIZARD

To launch an experiment search tool wizard, the user selects an experiment. Typically, selection of an experiment comprises (i) clicking on the experiment when it is displayed in a biological viewer and then (ii) either selecting a launch button, such as button 424 (FIG. 4), or activating a context-sensitive menu and selecting a biological comparison command. In one embodiment, the experiment search tool wizard comprises a series of successive screens, such as screens 2700 (FIG. 27), 2800 (FIG. 28), and 2900 (FIG. 29). In such an embodiment, the experiment search tool wizard automatically determines the species data warehouse in which the selected experiment resides. Typically, the species data warehouse is stored in biological response database 312 (FIG. 3). However, it will be appreciated that the term species data warehouse, hereafter called a "target database," is to be broadly construed and includes any experimental data that is reducible to machine readable form. Any such form of data is a "target database" and is within the scope of the present invention.

In screen 2700, the user is given the option to select whether or not the search is to be targeted to a subset of experiments in target database 2702. When the user elects to

search the entire target database, screen 2800 is skipped. When the user elects to search a subset of the target database, FINISH button 2704 is inactivated. Screen 2700 also provides the user with the option to refine the search to a subset of genes that are in the target database (2706). As shown in Figure 27, option 2706 allows a user to either select a search using all genes on the query experiment, which is the system default, or a search using a subset of genes from the query experiment. When the user selects the latter option, FINISH button 2704 is inactivated.

Screen 2700 further provides the user with an option to select datapoints that meet a P-value threshold (2708). In a non-limiting example, by clicking toggle 2708, the user may designate a P-value threshold of 0.10, 0.05 or 0.01. Further, by adjusting the toggle, the user may designate that the P-value threshold is to be applied to (i) the query, (ii) the target, or (iii) both the query and the target. Screen 2700 further provides an option to hide results of the search that were computed using fewer than a cutoff number (N) of datapoints (2712). Finally, screen 2700 provides the user with the option to limit the number of results returned to the X1 experiments that are most correlated with the query experiment (X1) and the Y1 experiments that are most anti-correlated with the target experiment (Y1) (2714). However, if the checkbox corresponding to 2714 is not selected, all results will be returned. When a user selects an X1 and a Y1, the settings X1 and Y1 are permanently stored by biological analysis software 302 as user preferences. However, in one embodiment, if the values X1 and Y1 are not available in the user's preferences, X1 will default to 30 and Y1 will default to 0.

When the user has entered preferred settings for 2702 thru 2714, the user presses NEXT key 2718 and screen 2700 is terminated. If the user has indicated that a subset of the experiments in the target database is to be searched, screen 2800 (FIG. 28) will appear. Screen 2800 lists a set of experiments 2802 that are present in the selected target database. Specifically, the user is provided with the option to search only the listed experiments 2802, or to search all experiments except the listed experiments 2802. The user may delete experiments 2802 from the list. However, the user is not allowed to add experiments 2802 that are not in the selected target database.

When the user has selected experiments 2802 that are to form the target, the user may need to continue to screen 2900 (FIG. 29) if 2706 (FIG. 27) has been toggled so that the search is restricted to particular genes. This is accomplished by pressing NEXT key 2804. However, if 2706 has not been toggled to restrict the search to specific genes, the biological comparison algorithm may be initiated by pressing FINISH key 2806.

Screen 2900 (FIG. 29) lists each of the genes 2902 that are present in the selected target experiments. Option 2904 allows the user to search only the listed genes 2902 or to

search all genes except the listed genes 2902 (2904). The user may add or delete individual genes 2902 on the list. Once target genes have been selected, the biological search algorithm can be launched by pressing FINISH key 2906.

### 5.1.2.2 GENE SEARCH TOOL WIZARD

One embodiment of gene search tool wizard is represented by screens 3000 (FIG. 30), 3100 (FIG. 31), and 3200 (FIG. 32). When a gene search tool wizard is launched, the user is first presented with screen 3000. The gene search tool wizard may be launched in many different ways from biological analysis software module 304 and/or biological analysis software module 302. For example, a gene search tool wizard could be a command in a context-sensitive menu. In such an example, when a user selects a gene that is displayed in a biological viewer, perhaps by right-clicking on the gene, a context sensitive menu is launched. One of the commands in the context sensitive menu initiates a biological comparison algorithm using the selected gene as a query.

Screen 3000 provides the user with an option to select the target database that is to be searched (3002). Advantageously, only those target databases that include the selected gene are listed (3004). Screen 3000 further provides option 3006, which allows the user to define a subset of members (experiments) in the target database 3002 that will be used in the comparison. When the user chooses to define a subset of experiments, FINISH key 3008 will be inactivated and the user will need to review screens 3100 and/or 3200 before the search can be initiated. As shown in FIG. 30, option 3006 provides the user with the option to search all genes in the target database or to search against a subset of genes in the target database.

Additionally, screen 3000 provides the user with option 3008. Option 3008 allows the user to refine the search to a subset of genes in the selected target database. When the user chooses to define a subset of genes to be used in the search, FINISH key 3008 button will be inactivated and the user will need to review screens 3100 (FIG. 31) and/or 3200 (FIG. 32) before the search can be launched. Screen 3000 also allows the user to indicate a threshold P-value 3010 that may be used to filter the target or query data. By clicking toggle 3010, the user may designate a P-value of 0.10, 0.05 or 0.01. Further, by adjusting the toggle, the user may designate that the P-value threshold is to be applied to (i) the query, (ii) the target, or (iii) both the query and the target. In one embodiment, a threshold P-value of 0.10 is used when the user indicates that a P-value should be used but does not, in fact, select a particular threshold P-value. When the user does not indicate that a threshold P-value is to be used, all data is used.

Screen 3000 further provides an option to hide results from the search that have less than a cutoff number (N) of datapoints 3012. When the user does not indicate a cutoff number, no datapoint cutoff is performed. Finally, screen 3000 provides the user with the option to limit the number of results returned to the X2 genes that are most correlated with the query gene (X2) and the Y2 genes that are most anti-correlated with the query gene (Y2) (2714). However, if the checkbox corresponding to 3014 is not selected, all results will be returned. When a user selects an X2 and a Y2, the settings X2 and Y2 are stored by biological analysis software 302 as user preferences. However, in one embodiment, if the values X2 and Y2 are not available in the user's preferences, X2 will default to 30 and Y2 will default to 0.

When the user has entered preferred settings for 3002 thru 3014, the user presses NEXT key 3016 and screen 3000 is terminated. If the user has indicated that a subset of the genes in the target database are to be searched, screen 3100 (FIG. 31) will appear. Screen 3100 lists each of the genes 3102 that are present in the target database. Specifically, the user is provided with the option to search using only genes 3102, or to search using all genes except listed genes 3102. The user may delete genes 3102 from the list. However, in the preferred embodiments, the user is not allowed to add genes 3102 that are not in the target database.

When the user has selected genes 3102 that are to form the target, the user may need to continue to screen 3200 (FIG. 32) if 3008 (FIG. 30) has been toggled to require that the search be restricted to certain experiments. This is accomplished by pressing NEXT key 3104. However, if 3008 has not been toggled to restrict the search to specific experiments, the search is initiated by pressing FINISH key 3106.

Referring to FIG. 32, screen 3200 lists each of the experiments 3202 that are associated with the selected target genes. Option 3204 allows the user to search only the listed experiments 3202 or to search all experiments except the listed experiments 3202. The user may add or delete experiments on the list. However, in one embodiment, the user is not permitted to add experiments that do not include a target gene selected in screens 3000 and/or 3100. Once target experiments have been selected, the biological search algorithm can be launched by pressing FINISH button 3206.

### 5.1.2.3 INFORMATION GATHERED BY SEARCH TOOL WIZARDS

In one embodiment, the search tool wizards of the present invention gather specific data necessary to initiate a search. Data gathered in such embodiments include, but are not limited to, (i) an indication that the query is a gene or an experiment, (ii) a unique identifier corresponding to the query, such as E_EXPERIMENT.EXPERIMENT_ID or

A_GENE.GENE_ID, (iii) a database identifier that specifies a specific target database, (iv) a P-value cutoff value, (v) a logical operator that indicates how the P-value is to applied (for example, to the target, the query, or both), (vi) an optional listing of a subset of experiments in the target database, (vii) an include/exclude flag to be applied against the subset experiments, (viii) an optional listing of a subset of genes in the target database, (ix) an include/exclude flag to be applied against the subset of genes, (x) a number of most highly correlated hits to be returned by the search, (xi) a number of most highly anti-correlated hits to be returned by the search, (xii) an identifier that defines the structure (data type) of the search results, and (xiii) a reference to an object that may be used to report search progress once the search is initiated.

## 5.2. MEASUREMENT METHODS

Biological response signals may be obtained for use in the invention in any number of ways, including by measurement of the change in cellular constituents, *e.g.*, after drug exposure or pathway perturbation. These cellular constituents can be of any aspect of the biological state of a cell. They can be, *e.g.*, of the transcription state, in which RNA abundances are measured, the translation state, in which protein abundances are measured, the activity state, in which protein activities are measured. The cellular characteristics can also be of mixed aspects, for example, in which the activities of one or more proteins originating a particular biological pathway are measured along with RNA abundances (gene expression) of cellular constituents in the pathway downstream of the originating protein(s). This section describes exemplary methods for measuring the cellular constituents in drug or pathway responses. This invention is adaptable to other methods of such measurement.

Embodiments of this invention based on measuring the transcriptional state of drug and pathway responses are preferred. The transcriptional state can be measured by techniques of hybridization to arrays of nucleic acid or nucleic acid mimic probes, described in the next subsection, or by other gene expression technologies, described in the subsequent subsection. However measured, the result is response data including values representing RNA abundance ratios, which usually reflect DNA expression ratios (in the absence of differences in RNA degradation rates). Such measurement methods are described in Section 5.4.2.

In various alternative embodiments of the present invention, aspects of the biological state other than the transcriptional state, such as the translational state, the activity state, or mixed aspects can be measured. Details of these embodiments are described in this section. Such measurement methods are described in Section 5.4.3.

### 5.2.1. MEASUREMENT OF DRUG RESPONSE DATA

To measure drug response data, cell are exposed to graded levels of the drug or drug candidate of interest. When the cells are grown *in vitro*, the compound is usually added to their nutrient medium. In the case of yeast, such as *S. cerevisiae*, it is preferably to harvest the cells in early log phase, since expression patterns are relatively insensitive to time of harvest at that time. The drug is added in a graded amount that depends on the particular characteristics of the drug, but usually will be between about 1 ng/ml and 100 mg/ml. In some cases a drug will be solubilized in a solvent such as DMSO.

The biological state of cells exposed to the drug and cells not exposed to the drug is measured according to any of the below described methods. Preferably, transcript or microarrays are used to find the mRNAs with altered expression due to exposure to the drug. However, other aspects of the biological state may also be measured to determine, *e.g.*, proteins with altered translation or activities due to exposure to the drug.

It is preferable for measurements of drug responses, in the case of two-colored differential hybridization described below, to measure also with reversed labeling. Also, it is preferable that the levels of drug exposure used provide sufficient resolution of rapidly changing regions of the drug response, *e.g.*, by using approximately ten levels of drug exposure.

### 5.2.2. TRANSCRIPTIONAL STATE MEASUREMENT

In general, measurement of the transcriptional state can be performed using any probe or probes which comprise a polynucleotide sequence and which are immobilized to a solid support or surface. For example, the probes may comprise DNA sequences, RNA sequences, or copolymer sequences of DNA and RNA. The polynucleotide sequences of the probes may also comprise DNA and/or RNA analogues, or combinations thereof. For example, the polynucleotide sequences of the probe may be full or partial sequences of genomic DNA, cDNA, or mRNA sequences extracted from cells. The polynucleotide sequences of the probes may also be synthesized nucleotide sequences, such as synthetic oligonucleotide sequences. The probe sequences can be synthesized either enzymatically *in vivo*, enzymatically *in vitro*, (*e.g.*, by PCR), or non-enzymatically *in vitro*.

The probe or probes used in the methods of the invention are preferably immobilized to a solid support or surface which may be either porous or non-porous. For example, the probes of the invention may be polynucleotide sequences which are attached to a nitrocellulose or nylon membrane or filter. Such hybridization probes are well known in the art (see, *e.g.*, Sambrook *et al.*, Eds., 1989, *Molecular Cloning: A Laboratory*

hybridize. The DNA or DNA analogue can be, *e.g.*, a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

Although in a preferred embodiment the microarray contains binding sites for products of all or almost all genes in the target organism's genome, such comprehensiveness is not necessarily required. Usually the microarray will have binding sites corresponding to at least about 50% of the genes in the genome, often to about 75%, more often to at least about 85%, even more often to about 90%, and still more often to at least about 99%. Preferably, the microarray has binding sites for genes relevant to the action of a drug of interest or in a biological pathway of interest. A "gene" is identified as an open reading frame ("ORF") which encodes a sequence of preferably at least 50, 75, or 99 amino acids from which a messenger RNA is transcribed in the organism or in some cell in a multicellular organism. The number of genes in a genome can be estimated from the number of mRNAs expressed by the organism, or by extrapolation from a well characterized portion of the genome. When the genome of the organism of interest has been sequenced, the number of ORF's can be determined and mRNA coding regions identified by analysis of the DNA sequence. For example, the genome of *Saccharomyces cerevisiae* has been completely sequenced, and is reported to have approximately 6275 ORFs longer than 99 amino acids. Analysis of the ORFs indicates that there are 5885 ORFs that are likely to encode protein products (Goffeau *et al.*, 1996, *Science 274*:546-567). In contrast, the human genome is estimated to contain approximately $10^5$ genes.

### 5.2.2.2. PREPARING PROBES FOR MICROARRAYS

As noted above, the "probe" to which a particular polynucleotide molecule specifically hybridizes according to the invention is usually a complementary polynucleotide sequence. In one embodiment, the probes of the microarray are DNA or DNA "mimics" (*e.g.*, derivatives and analogues) corresponding to at least a portion of each gene in an organism's genome. In another embodiment, the probes of the microarray are complementary RNA or RNA mimics.

DNA mimics are polymers composed of subunits capable of specific, Watson-Crick-like hybridization with DNA, or of specific hybridization with RNA. The nucleic acids can be modified at the base moiety, at the sugar moiety, or at the phosphate backbone. Exemplary DNA mimics include, *e.g.*, phosphorothioates.

DNA can be obtain, *e.g.*, by polymerase chain reaction ("PCR") amplicafication of gene segments from genomic DNA, cDNA (*e.g.*, by RT-PCR), or clones sequences. PCR primers are preferably chosen based on known sequences of the genes or cDNA that result in amplification of unique fragments (*i.e.g*, fragments that do not share more than 10 bases

of contiguous identical sequence with any other fragment on the microarray). Computer programs that are well known in the art are useful in the design of primer with the required specificity and optimal amplification properties, such as *Oligo* version 5.0 (National Biosciences). Typically, each probe of the microarray will be between about 20 bases and about 12,000 bases, and usually between about 300 bases and about 2,000 bases in length, and still more usually between about 300 bases and about 800 bases in length. PCR methods are well known in the art, and are described, for example, in Innis *et al.*, eds., 1990, *PCR Protocols: A Guide to Methods and Applications*, Academic Press Inc., San Diego, CA. It will be apparent to one skilled in the art that controlled robotic systems are useful for isolating and amplifying nucleic acids.

An alternative means for generating the polynucleotide probes of the microarray is by synthesis of synthetic polynucleotides or oligonucleotides, *e.g.*, using N-phosphonate or phosphoramidite chemistries (Froehler *et al.*, 1986, *Nucleic Acid Res. 14*:5399-5407; McBrid *et al.*, 1983, *Tetrahedron Lett. 24*:246-248). Synthetic sequences are typically between about 15 and about 500 bases in length, more typically between about 20 and about 50 bases. In some embodiments, synthetic nucleic acids include non-natural bases, such as, but by no means limited to, inosine. As noted above, nucleic acid analogues may be used as binding sites for hybridization. An example of a suitable nucleic acid analogue is peptide nucleic acid (see, *e.g.*, Egholm *et al.*, 1993, *Nature 363*:566-568; U.S. Patent No. 5,539,083).

In alternative embodiments, the hybridization sites (*i.e.*, the probes) are made from plasmid or phage clones of genes, cDNAs (*e.g.*, expressed sequence tags), or inserts therefrom (Nguyen *et al.*, 1995, *Genomics 29*:207-209).

#### 5.2.2.3. ATTACHING PROBES TO THE SOLID SURFACE

The probes are attached to a solid support or surface, which may be made, *e.g.*, from glass, plastic (*e.g.*, polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena *et al.*, 1995, *Science 270*:467-470. This method is especially useful for preparing microarrays of cDNA (See also, DeRisi *et al.*, 1996, *Nature Genetics 14*:457-460; Shalon *et al.*, 1996, *Genome Res. 6*:689-645; and Schena *et al.*, 1995, *Proc. Natl. Acad. Sci. U.S.A. 93*:10539-11286). Blanchard discloses the use of an ink jet printer for oligonucleotide synthesis (WO98/41531 dated Sept. 24, 1998).

A second preferred method for making microarrays is by making high-density oligonucleotide arrays. Techniques are known for producing arrays containing thousands of

oligonucleotides complementary to defined sequences, at defined locations on a surface using photolithographic techniques for synthesis *in situ* (see, Fodor *et al.*, 1991, *Science 251*:767-773; Pease *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A. 91*:5022-5026; Lockhart *et al.*, 1996, *Nature Biotechnology 14*:1675; U.S. Patent Nos. 5,578,832; 5,556,752; and 5,510,270) or other methods for rapid synthesis and deposition of defined oligonucleotides (Blanchard *et al.*, *Biosensors & Bioelectronics 11*:687-690). When these methods are used, oligonucleotides (*e.g.*, 20-mers) of known sequence are synthesized directly on a surface such as a derivatized glass slides. Usually, the array produced is redundant, with several oligonucleotide molecules per RNA. Oligonucleotide probes can be chosen to detect alternatively spliced mRNAs.

Other methods for making microarrays, *e.g.*, by masking (Maskos and Southern, 1992, *Nuc. Acids. Res. 20*:1679-1684), may also be used. In principle, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook *et al.*, *supra*) could be used. However, as will be recognized by those skilled in the art, very small arrays will frequently be preferred because hybridization volumes will be smaller.

### 5.2.2.4. TARGET POLYNUCLEOTIDE MOLECULES

As described, *supra*, the polynucleotide molecules which may be analyzed by the present invention may be from any source, including naturally occurring nucleic acid molecules, as well as synthetic nucleic acid molecules. In a preferred embodiment, the polynucleotide molecules analyzed by the invention comprise RNA, including, but by no means limited to, total cellular RNA, poly(A)$^+$ messenger RNA (mRNA), fractions thereof, or RNA transcribed from cDNA. Methods for preparing total and poly(A)$^+$ RNA are well known in the art, and are described generally, *e.g.*, in Sambrook *et al.*, *supra*. In one embodiment, RNA is extracted from cells of the various types of interest in this invention using guanidinium thiocyanate lysis followed by CsCl centrifugation (Chirgwin *et al.*, 1979, *Biochemistry 18*:5294-5299). Poly (A)$^+$ RNA is selected by selection with oligo-dT cellulose. Cells of interest include, but are by no means limited to, wild-type cells, drug-exposed wild-type cells, modified cells, diseased cells, and, in particular, cancer cells.

In one embodiment, RNA can be fragmented by methods known in the art, *e.g.*, by incubation with $ZnCl_2$, to generate fragments of RNA. In one embodiment, isolated mRNA can be converted to antisense RNA synthesized by *in vitro* transcription of double-stranded cDNA in the presence of labeled dNTPs (Lockhart *et al.*, 1996, *Nature Biotechnology 14*:1675).

In other embodiments, the polynucleotide molecules to be analyzed may be DNA molecules such as fragmented genomic DNA, first strand cDNA which is reverse transcribed from mRNA, or PCR products of amplified mRNA or cDNA.

#### 5.2.2.5. HYBRIDIZATION TO MICROARRAYS

Nucleic acid hybridization and wash conditions are chosen so that the polynucleotide molecules to be analyzed by the invention "specifically bind" or "specifically hybridize" to the complementary polynucleotide sequences of the array, preferably to a specific array site, wherein its complementary DNA is located.

Arrays containing double-stranded probe DNA situated thereon are preferably subjected to denaturing conditions to render the DNA single-stranded prior to contacting with the target polynucleotide molecules. Arrays containing single-stranded probe DNA (*e.g.*, synthetic oligodeoxyribonucleic acids) may need to be denatured prior to contacting with the target polynucleotide molecules, *e.g.*, to remove hairpins or dimers which form due to self complementary sequences.

Optimal hybridization conditions will depend on the length (*e.g.*, oligomer versus polynucleotide greater than 200 bases) and type (*e.g.*, RNA or DNA) of probe and target nucleic acids. General parameters for specific (*i.e.*, stringent) hybridization conditions are described in Sambrook *et al.* (*supra*), and in Ausubel *et al.*, 1987, *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York. When the cDNA microarrays of Schena *et al.* are used, typical hybridization conditions are hybridization in 5x SSC plus 0.2% SDS at 65 °C for four hours, followed by washes at 25 °C in high stringency wash buffer (0.1x SSC plus 0.2% SDS) (Shena *et al.*, 1996, *Proc. Natl. Acad. Sci. U.S.A. 93*:10614). Useful hybridization conditions are also provided, *e.g.*, Tijessen, 1993, *Hybridization With Nucleic Acid Probes*, Elsevier Science Publishers B.V.; and Kricka, 1992, *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego, CA.

#### 5.2.2.6. SIGNAL DETECTION AND DATA ANALYSIS

It will be appreciated that when cDNA complementary to the RNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level of hybridization to the site in the array corresponding to any particular gene will reflect the prevalence in the cell of mRNA transcribed from that gene. For example, when detectably labeled (*e.g.*, with a fluorophore) cDNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to a gene (*i.e.*, capable of specifically binding the product of the gene) that is not transcribed in the cell will have little

or no signal (*e.g.*, fluorescent signal), and a gene for which the encoded mRNA is prevalent will have a relatively strong signal.

In preferred embodiments, cDNAs from two different cells are hybridized to the binding sites of the microarray. In the case of drug responses, one cell is exposed to a drug and another cell of the same type is not exposed to the drug. The cDNA derived from each of the two cell types are differently labeled so that they can be distinguished. In one embodiment, for example, cDNA from a cell treated with a drug is synthesized using a fluorescein-labeled dNTP, and cDNA from a second cell, not drug-exposed, is synthesized using a rhodamine-labeled dNTP. When the two cDNAs are mixed and hybridized to the microarray, the relative intensity of signal from each cDNA set is determined for each site on the array, and any relative difference in abundance of a particular mRNA is thereby detected.

In the example described above, the cDNA from the drug-treated cell will fluoresce green when the fluorophore is stimulated, and the cDNA from the untreated cell will fluoresce red. As a result, when the drug treatment has no effect, either directly or indirectly, on the relative abundance of a particular mRNA in a cell, the mRNA will be equally prevalent in both cells, and, upon reverse transcription, red-labeled and green-labeled cDNA will be equally prevalent. When hybridized to the microarray, the binding site(s) for that species of RNA will emit wavelength characteristic of both fluorophores. In contrast, when the drug-exposed cell is treated with a drug that, directly or indirectly, increases the prevalence of the mRNA in the cell, the ratio of green to red fluorescence will increase. When the drug decreases the mRNA prevalence, the ratio will decrease.

The use of a two-color fluorescence labeling and detection scheme to define alterations in gene expression has been described, *e.g.*, in Shena *et al.*, 1995, *Science 270*:467-470. An advantage of using cDNA labeled with two different fluorophores is that a direct and internally controlled comparison of the mRNA levels corresponding to each arrayed gene in two cell states can be made, and variations due to minor differences in experimental conditions (*e.g.*, hybridization conditions) will not affect subsequent analyses. However, it will be recognized that it is also possible to use cDNA from a single cell, and compare, for example, the absolute amount of a particular mRNA in, *e.g.*, a drug-treated or pathway-perturbed cell and an untreated cell.

When fluorescently labeled probes are used, the fluorescence emissions at each site of a transcript array can be, preferably, detected by scanning confocal laser microscopy. In one embodiment, a separate scan, using the appropriate excitation line, is carried out for each of the two fluorophores used. Alternatively, a laser can be used that allows simultaneous specimen illumination at wavelengths specific to the two fluorophores and

emissions from the two fluorophores can be analyzed simultaneously (see Shalon *et al.*, 1996, *Genome Res.* 6:639-645). In a preferred embodiment, the arrays are scanned with a laser fluorescent scanner with a computer controlled X-Y stage and a microscope objective. Sequential excitation of the two fluorophores is achieved with a multi-line, mixed gas laser, and the emitted light is split by wavelength and detected with two photomultiplier tubes. Such fluorescence laser scanning devices are described, *e.g.*, in Schena *et al.*, 1996, *Genome Res.* 6:639-645. Alternatively, the fiber-optic bundle described by Ferguson *et al.*, 1996, *Nature Biotech.* 14:1681-1684, may be used to monitor mRNA abundance levels at a large number of sites simultaneously.

Signals are recorded and, in a preferred embodiment, analyzed by computer, *e.g.*, using a 12 bit analog to digital board. In one embodiment, the scanned image is despeckled using a graphics program (*e.g.*, Hijaak Graphics Suite) and then analyzed using an image gridding program that creates a spreadsheet of the average hybridization at each wavelength at each site. If necessary, an experimentally determined correction for "cross talk" (or overlap) between the channels for the two fluors may be made. For any particular hybridization site on the transcript array, a ratio of the emission of the two fluorophores can be calculated. The ratio is independent of the absolute expression level of the cognate gene, but is useful for genes whose expression is significantly modulated by drug administration, gene deletion, or any other tested event.

According to the method of the invention, the relative abundance of an mRNA in two cells or cell lines is scored as a perturbation and its magnitude determined (*i.e.*, the abundance is different in the two sources of mRNA tested) or as not perturbed (*i.e.*, the relative abundance is the same). As used herein, a difference between the two sources of RNA of at least a factor of about 25% (*i.e.*, RNA is 25% more abundant in one source than in the other source), more usually about 50%, even more often by a factor of about 2 (*i.e.*, twice as abundant), 3 (three times as abundant), or 5 (five times as abundant) is scored as a perturbation. Present detection methods allow reliable detection of difference of an order of about 3-fold to about 5-fold, but more sensitive methods are expected to be developed.

Preferably, in addition to identifying a perturbation as positive or negative, it is advantageous to determine the magnitude of the perturbation. This can be carried out, as noted above, by calculating the ratio of the emission of the two fluorophores used for differential labeling, or by analogous methods that will be readily apparent to those of skill in the art.

#### 5.2.2.7. OTHER METHODS OF TRANSCRIPTIONAL STATE MEASUREMENT

The transcriptional state of a cell may be measured by other gene expression technologies known in the art. Several such technologies produce pools of restriction fragments of limited complexity for electrophoretic analysis, such as methods combining double restriction enzyme digestion with phasing primers (see, *e.g.*, European Patent O 534858 A1, filed September 24, 1992, by Zabeau *et al.*), or methods selecting restriction fragments with sites closest to a defined mRNA end (see, *e.g.*, Prashar *et al.*, 1996, *Proc. Natl. Acad. Sci. U.S.A. 93*:659-663). Other methods statistically sample cDNA pools, such as by sequencing sufficient bases (*e.g.*, 20-50 bases) in each of multiple cDNAs to identify each cDNA, or by sequencing short tags (*e.g.*, 9-10 bases) which are generated at known positions relative to a defined mRNA end (see, *e.g.*, Velculescu, 1995, *Science 270*:484-487).

Such methods and systems of measuring transcriptional state, although less preferable than microarrays, may, nevertheless, be used in the present invention.

### 5.2.3. MEASUREMENTS OF OTHER ASPECTS OF BIOLOGICAL STATE

Although monitoring cellular constituents other than mRNA abundances currently presents certain technical difficulties not encountered in monitoring mRNAs (*i.e.*, the transcriptional state), it will be apparent to those skilled in the art that the use of methods of this invention are applicable to any cellular constituent that can be monitored.

In various embodiments of the present invention, aspects of the biological state other than the transcriptional state, such as the translational state, the activity state, or mixed aspects thereof can be measured in order to obtain drug responses for the present invention. Details of these embodiments are described in this section.

#### 5.2.3.1. TRANSLATIONAL STATE MEASUREMENTS

Measurements of the translational state may be performed according to several methods. For example, whole genome monitoring of protein (*i.e.*, the "proteome," Goffea *et al.*, *supra*) can be carried out by constructing a microarray in which binding sites comprise immobilized, preferably monoclonal, antibodies specific to a plurality of protein species encoded by the cell genome. Preferably, antibodies are present for a substantial fraction of the encoded proteins, or at least for those proteins relevant to the action of a drug of interest. Methods for making monoclonal antibodies are well known (see, *e.g.*, Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, New York). In a preferred embodiment, monoclonal antibodies are raised against synthetic peptide fragments designed based on genomic sequence of the cell. With such an antibody array, proteins

from the cell are contacted to the array, and their binding is assayed with assays known in the art.

Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well known in the art, and typically involves iso-electric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. See, *e.g.*, Hames *et al.*, 1990, *Gel Electrophoresis of Proteins: A Practical Approach*, IRL Press, New York; Shevchenko *et al.*, 1996, *Proc. Natl. Acad. Sci. U.S.A. 93*:1440-1445; Sagliocco *et al.*, 1996, *Yeast 12*:1519-1533; and Lander, 1996, *Science 274*:536-539. The resulting electropherograms can be analyzed by numerous techniques, including mass spectrometric techniques, western blotting, and immunoblot analysis using polyclonal and monoclonal antibodies, and internal and N-terminal micro-sequencing. Using these techniques, it is possible to identify a substantial fraction of all the proteins produced under given physiological conditions, including in cells (*e.g.*, in yeast) exposed to a drug, or in cells modified by, *e.g.*, deletion or over-expression of a specific gene.

### 5.2.3.2. ACTIVITY STATE MEASUREMENTS

Where activities of proteins relevant to the characterization of drug action can be measured, embodiments of this invention can be based on such measurements. Activity measurements can be performed by any functional, biochemical, or physical means appropriate to the particular activity being characterized. Where the activity involves a chemical transformation, the cellular protein can be contacted with the natural substrate(s), and the rate of transformation measured. Where the activity involves association in multimeric units, for example association of an activated DNA binding complex with DNA, the amount of associated protein or secondary consequences of the association, such as amounts of mRNA transcribed, can be measured. Also, where only a functional activity is known, for example, as in cell cycle control, performance of the function can be observed. However known or measured, the changes in protein activities form the response data analyzed by the foregoing methods of this invention.

### 5.2.3.3. MIXED ASPECTS OF BIOLOGICAL STATE

In alternative and non-limiting embodiments, response data may be formed of mixed aspects of the biological state of a cell. Response data can be constructed from combinations of, *e.g.*, changes in certain mRNA abundances, changes in certain protein abundances, and changes in certain protein activities.

## 5.3. TARGETED PERTURBATION METHODS

Methods for targeted perturbation of biological pathways at various levels of a cell are increasingly widely known and applied in the art. Any such methods that are capable of specifically targeting and controllably modifying (*e.g.*, either by a graded increase or activation or by a graded decrease or inhibition) specific cellular constituents (*e.g.*, gene expression, RNA concentrations, protein abundances, protein activities, or so forth) can be employed in performing pathway perturbations. Controllable modifications of cellular constituents consequentially controllably perturb pathways originating at the modified cellular constituents. Such pathways originating at specific cellular constituents are preferably employed to represent drug action in this invention. Preferable modification methods are capable of individually targeting each of a plurality of cellular constituents and most preferably a substantial fraction of such cellular constituents.

The following methods are exemplary of those that can be used to modify cellular constituents and thereby to produce pathway perturbations which generate the pathway responses used in the steps of the methods of this invention as previously described. This invention is adaptable to other methods for making controllable perturbations to pathways, and especially to cellular constituents from which pathways originate.

Pathway perturbations are preferably made in cells of cell types derived from any organism for which genomic or expressed sequence information is available and for which methods are available that permit controllably modification of the expression of specific genes. Genome sequencing is currently underway for several eukaryotic organisms, including humans, nematodes, *Arabidopsis*, and flies. In a preferred embodiment, the invention is carried out using a yeast, with *Saccharomyces cerevisiae* most preferred because the sequence of the entire genome of a *S. cerevisiae* strain has been determined. In addition, well-established methods are available for controllably modifying expression of year genes. A preferred strain of yeast is a *S. cerevisiae* strain for which yeast genomic sequence is known, such as strain S288C or substantially isogeneic derivatives of it (*see, e.g.*, Dujon *et al.*, 1994, *Nature* 369:371-378; Bussey *et al.*, 1995, *Proc. Natl. Acad. Sci. U.S.A. 92*:3809-3813; Feldmann *et al.*, 1994, *E.M.B.O. J. 13*:5795-5809; Johnston *et al.*, 1994, *Science 265*:2077-2082; Galibert *et al.*, 1996, *E.M.B.O. J. 15*:2031-2049). However, other strains may be used as well. Yeast strains are available, *e.g.*, from American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209. Standard techniques for manipulating yeast are described in C. Kaiser, S. Michaelis, & A. Mitchell, 1994, *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, New York; and Sherman *et al.*, 1986, *Methods in Yeast*

*Genetics: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor. New York.

The exemplary methods described in the following include use of titratable expression systems, use of transfection or viral transduction systems, direct modifications to RNA abundances or activities, direct modifications of protein abundances, and direct modification of protein activities including use of drugs (or chemical moieties in general) with specific known action.

### 5.3.1. TITRATABLE EXPRESSION SYSTEMS

Any of the several known titratable, or equivalently controllable, expression systems available for use in the budding yeast *Saccharomyces cerevisiae* are adaptable to this invention (Mumberg et al., 1994, *Nucl. Acids Res. 22*:5767-5768). Usually, gene expression is controlled by transcriptional controls, with the promoter of the gene to be controlled replaced on its chromosome by a controllable, exogenous promoter. The most commonly used controllable promoter in yeast is the GAL1 promoter (Johnston *et al.*, 1984, *Mol Cell. Biol. 8*:1440-1448). The GAL1 promoter is strongly repressed by the presence of glucose in the growth medium, and is gradually switched on in a graded manner to high levels of expression by the decreasing abundance of glucose and the presence of galactose. The GAL1 promoter usually allows a 5-100 fold range of expression control on a gene of interest.

Other frequently used promoter systems include the MET25 promoter (Kerjan *et al.*, 1986, *Nucl. Acids. Res. 14*:7861-7871), which is induced by the absence of methionine in the growth medium, and the CUP1 promoter, which is induced by copper (Mascorro-Gallardo et al., 1996, *Gene 172*:169-170). All of these promoter systems are controllable in that gene expression can be incrementally controlled by incremental changes in the abundances of a controlling moiety in the growth medium.

One disadvantage of the above listed expression systems is that control of promoter activity (effected by, *e.g.*, changes in carbon source, removal of certain amino acids), often causes other changes in cellular physiology which independently alter the expression levels of other genes. A recently developed system for yeast, the Tet system, alleviates this problem to a large extent (Gari et al., 1997, *Yeast 13*:837-848). The Tet promoter, adopted from mammalian expression systems (Gossen et al., 1995, *Proc. Nat. Acad. Sci. USA 89*:5547-5551) is modulated by the concentration of the antibiotic tetracycline or the structurally related compound doxycycline. Thus, in the absence of doxycycline, the promoter induces a high level of expression, and the addition of increasing levels of doxycycline causes increased repression of promoter activity. Intermediate levels gene

expression can be achieved in the steady state by addition of intermediate levels of drug. Furthermore, levels of doxycycline that give maximal repression of promoter activity (10 micrograms/ml) have no significant effect on the growth rate on wild type yeast cells (Gari et al., 1997, *Yeast 13*:837-848).

In mammalian cells, several means of titrating expression of genes are available (Spencer, 1996, *Trends Genet. 12*:181-187). As mentioned above, the Tet system is widely used, both in its original form, the "forward" system, in which addition of doxycycline represses transcription, and in the newer "reverse" system, in which doxycycline addition stimulates transcription (Gossen et al., 1995, *Proc. Natl. Acad. Sci. USA 89*:5547-5551; Hoffmann et al., 1997, *Nucl. Acids. Res. 25*:1078-1079; Hofmann et al., 1996, *Proc. Natl. Acad. Sci. USA 83*:5185-5190; Paulus et al., 1996, *Journal of Virology 70*:62-67). Another commonly used controllable promoter system in mammalian cells is the ecdysone-inducible system developed by Evans and colleagues (No et al., 1996, *Proc. Nat. Acad. Sci. USA 93*:3346-3351), where expression is controlled by the level of muristerone added to the cultured cells. Finally, expression can be modulated using the "chemical-induced dimerization" (CID) system developed by Schreiber, Crabtree, and colleagues (Belshaw et al., 1996, *Proc. Nat. Acad. Sci. USA 93*:4604-4607; Spencer, 1996, *Trends Genet. 12*:181-187) and similar systems in yeast. In this system, the gene of interest is put under the control of the CID-responsive promoter, and transfected into cells expressing two different hybrid proteins, one comprised of a DNA-binding domain fused to FKBP12, which binds FK506. The other hybrid protein contains a transcriptional activation domain also fused to FKBP12. The CID inducing molecule is FK1012, a homodimeric version of FK506 that is able to bind simultaneously both the DNA binding and transcriptional activating hybrid proteins. In the graded presence of FK1012, graded transcription of the controlled gene is activated.

For each of the mammalian expression systems described above, as is widely known to those of skill in the art, the gene of interest is put under the control of the controllable promoter, and a plasmid harboring this construct along with an antibiotic resistance gene is transfected into cultured mammalian cells. In general, the plasmid DNA integrates into the genome, and drug resistant colonies are selected and screened for appropriate expression of the regulated gene. Alternatively, the regulated gene can be inserted into an episomal plasmid such as pCEP4 (Invitrogen, Inc.), which contains components of the Epstein-Barr virus necessary for plasmid replication.

In a preferred embodiment, titratable expression systems, such as the ones described above, are introduced for use into cells or organisms lacking the corresponding endogenous gene and/or gene activity, *e.g.*, organisms in which the endogenous gene has been disrupted

or deleted. Methods for producing such "knock outs" are well known to those of skill in the art, see *e.g.*, Pettitt *et al.*, 1996, *Development 122*:4149-4157; Spradling *et al.*, 1995, *Proc. Natl. Acad. Sci. USA, 92*:10824-10830; Ramirez-Solis *et al.*, 1993, *Methods Enzymol. 225*:855-878; and Thomas *et al.*, 1987, *Cell 51*:503-512.

### 5.3.2. TRANSFECTION SYSTEMS FOR MAMMALIAN CELLS

Transfection or viral transduction of target genes can introduce controllable perturbations in biological pathways in mammalian cells. Preferably, transfection or transduction of a target gene can be used with cells that do not naturally express the target gene of interest. Such non-expressing cells can be derived from a tissue not normally expressing the target gene or the target gene can be specifically mutated in the cell. The target gene of interest can be cloned into one of many mammalian expression plasmids, for example, the pcDNA3.1 +/- system (Invitrogen, Inc.) or retroviral vectors, and introduced into the non-expressing host cells. Transfected or transduced cells expressing the target gene may be isolated by selection for a drug resistance marker encoded by the expression vector. The level of gene transcription is monotonically related to the transfection dosage. In this way, the effects of varying levels of the target gene may be investigated.

A particular example of the use of this method is the search for drugs that target the src-family protein tyrosine kinase, lck, a key component of the T cell receptor activation pathway (Anderson *et al.*, 1994, *Adv. Immunol. 56*:171-178). Inhibitors of this enzyme are of interest as potential immunosuppressive drugs (Hanke JH, 1996, *J. Biol Chem 271(2)*:695-701). A specific mutant of the Jurkat T cell line (JcaM1) is available that does not express lck kinase (Straus *et al.*, 1992, *Cell 70*:585-593). Therefore, introduction of the lck gene into JCaM1 by transfection or transduction permits specific perturbation of pathways of T cell activation regulated by the lck kinase. The efficiency of transfection or transduction, and thus the level of perturbation, is dose related. The method is generally useful for providing perturbations of gene expression or protein abundances in cells not normally expressing the genes to be perturbed.

### 5.3.3. METHODS OF MODIFYING RNA ABUNDANCES OR ACTIVITIES

Methods of modifying RNA abundances and activities currently fall within three classes, ribozymes, antisense species, and RNA aptamers (Good *et al.*, 1997, *Gene Therapy 4*: 45-54). Controllable application or exposure of a cell to these entities permits controllable perturbation of RNA abundances.

Ribozymes are RNAs which are capable of catalyzing RNA cleavage reactions. (Cech, 1987, *Science 236*:1532-1539; PCT International Publication WO 90/11364,

published October 4, 1990; Sarver *et al.*, 1990, *Science 247*: 1222-1225). "Hairpin" and "hammerhead" RNA ribozymes can be designed to specifically cleave a particular target mRNA. Rules have been established for the design of short RNA molecules with ribozyme activity, which are capable of cleaving other RNA molecules in a highly sequence specific way and can be targeted to virtually all kinds of RNA. (Haseloff *et al.*, 1988, *Nature 334*:585-591; Koizumi *et al.*, 1988, *FEBS Lett. 228*:228-230; Koizumi *et al.*, 1988, *FEBS Lett. 239:285-288*). Ribozyme methods involve exposing a cell to, inducing expression in a cell, etc. of such small RNA ribozyme molecules. (Grassi and Marini, 1996, *Annals of Medicine 28*: 499-510; Gibson, 1996, *Cancer and Metastasis Reviews 15*: 287-299).

Ribozymes can be routinely expressed *in vivo* in sufficient number to be catalytically effective in cleaving mRNA, and thereby modifying mRNA abundances in a cell. (Cotten et al., 1989, *EMBO J. 8*:3861-3866). In particular, a ribozyme coding DNA sequence, designed according to the previous rules and synthesized, for example, by standard phosphoramidite chemistry, can be ligated into a restriction enzyme site in the anticodon stem and loop of a gene encoding a tRNA, which can then be transformed into and expressed in a cell of interest by methods routine in the art. Preferably, an inducible promoter (*e.g.*, a glucocorticoid or a tetracycline response element) is also introduced into this construct so that ribozyme expression can be selectively controlled. tDNA genes (*i.e.*, genes encoding tRNAs) are useful in this application because of their small size, high rate of transcription, and ubiquitous expression in different kinds of tissues. Therefore, ribozymes can be routinely designed to cleave virtually any mRNA sequence, and a cell can be routinely transformed with DNA coding for such ribozyme sequences such that a controllable and catalytically effective amount of the ribozyme is expressed. Accordingly the abundance of virtually any RNA species in a cell can be perturbed.

In another embodiment, activity of a target RNA (preferable mRNA) species, specifically its rate of translation, can be controllably inhibited by the controllable application of antisense nucleic acids. An "antisense" nucleic acid as used herein refers to a nucleic acid capable of hybridizing to a sequence-specific (*e.g.*, non-poly A) portion of the target RNA, for example its translation initiation region, by virtue of some sequence complementarity to a coding and/or non-coding region. The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered in a controllable manner to a cell or which can be produced intracellularly by transcription of exogenous, introduced sequences in controllable quantities sufficient to perturb translation of the target RNA.

Preferably, antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 200 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A. 86*: 6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci. U.S.A. 84*: 648-652; PCT Publication No. WO 88/09810, published December 15, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *BioTechniques 6*: 958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res. 5*: 539-549).

In a preferred aspect of the invention, an antisense oligonucleotide is provided, preferably as single-stranded DNA. The oligonucleotide may be modified at any position on its structure with constituents generally known in the art.

The antisense oligonucleotides may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is a 2-α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual ß-units, the strands run parallel to each other (Gautier *et al.*, 1987, *Nucl. Acids Res. 15*: 6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of a target RNA species. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a target RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex. The amount of antisense nucleic acid that will be effective in the inhibiting translation of the target RNA can be determined by standard assay techniques.

Oligonucleotides for use in the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (1988, *Nucl. Acids Res. 16*: 3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A. 85*: 7448-7451), etc. In another embodiment, the oligonucleotide is a 2'-0-methylribonucleotide (Inoue *et al.*, 1987, *Nucl. Acids Res. 15*: 6131-6148), or a chimeric RNA-DNA analog (Inoue *et al.*, 1987, *FEBS Lett. 215*: 327-330).

The synthesized antisense oligonucleotides can then be administered to a cell in a controlled manner. For example, the antisense oligonucleotides can be placed in the growth environment of the cell at controlled levels where they may be taken up by the cell. The uptake of the antisense oligonucleotides can be assisted by use of methods well known in the art.

In an alternative embodiment, the antisense nucleic acids of the invention are controllably expressed intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequences encoding the antisense RNAs can be by any promoter known in the art to act in a cell of interest. Such promoters can be inducible or constitutive. Most preferably, promoters are controllable or inducible by the administration of an exogenous moiety in order to achieve controlled expression of the antisense oligonucleotide. Such controllable promoters include the Tet promoter. Less preferably usable promoters for mammalian cells include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature 290*: 304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, *Cell 22*: 787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A. 78*: 1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, *Nature 296*: 39-42), etc.

Therefore, antisense nucleic acids can be routinely designed to target virtually any mRNA sequence, and a cell can be routinely transformed with or exposed to nucleic acids coding for such antisense sequences such that an effective and controllable amount of the antisense nucleic acid is expressed. Accordingly the translation of virtually any RNA species in a cell can be controllably perturbed.

Finally, in a further embodiment, RNA aptamers can be introduced into or expressed in a cell. RNA aptamers are specific RNA ligands for proteins, such as for Tat and Rev RNA (Good *et al.*, 1997, *Gene Therapy 4*: 45-54) that can specifically inhibit their translation.

### 5.3.4. METHODS OF MODIFYING PROTEIN ABUNDANCES

Methods of modifying protein abundances include, *inter alia*, those altering protein degradation rates and those using antibodies (which bind to proteins affecting abundances of activities of native target protein species). Increasing (or decreasing) the degradation rates of a protein species decreases (or increases) the abundance of that species. Methods for controllably increasing the degradation rate of a target protein in response to elevated

temperature and/or exposure to a particular drug, which are known in the art, can be employed in this invention. For example, one such method employs a heat-inducible or drug-inducible N-terminal degron, which is an N-terminal protein fragment that exposes a degradation signal promoting rapid protein degradation at a higher temperature (*e.g.*, 37° C) and which is hidden to prevent rapid degradation at a lower temperature (*e.g.*, 23° C) (Dohmen *et al.*, 1994, *Science 263*:1273-1276). Such an exemplary degron is Arg-DHFR$^{ts}$, a variant of murine dihydrofolate reductase in which the N-terminal Val is replaced by Arg and the Pro at position 66 is replaced with Leu. According to this method, for example, a gene for a target protein, P, is replaced by standard gene targeting methods known in the art (Lodish et al., 1995, *Molecular Biology of the Cell*, Chpt. 8, New York: W.H. Freeman and Co.) with a gene coding for the fusion protein Ub-Arg-DHFR$^{ts}$-P ("Ub" stands for ubiquitin). The N-terminal ubiquitin is rapidly cleaved after translation exposing the N-terminal degron. At lower temperatures, lysines internal to Arg-DHFR$^{ts}$ are not exposed, ubiquitination of the fusion protein does not occur, degradation is slow, and active target protein levels are high. At higher temperatures (in the absence of methotrexate), lysines internal to Arg-DHFR$^{ts}$ are exposed, ubiquitination of the fusion protein occurs, degradation is rapid, and active target protein levels are low. Heat activation of degradation is controllably blocked by exposure methotrexate. This method is adaptable to other N-terminal degrons which are responsive to other inducing factors, such as drugs and temperature changes.

Target protein abundances and also, directly or indirectly, their activities can also be decreased by (neutralizing) antibodies. By providing for controlled exposure to such antibodies, protein abundances/activities can be controllably modified. For example, antibodies to suitable epitopes on protein surfaces may decrease the abundance, and thereby indirectly decrease the activity, of the wild-type active form of a target protein by aggregating active forms into complexes with less or minimal activity as compared to the wild-type unaggregated wild-type form. Alternately, antibodies may directly decrease protein activity by, *e.g.*, interacting directly with active sites or by blocking access of substrates to active sites. Conversely, in certain cases, (activating) antibodies may also interact with proteins and their active sites to increase resulting activity. In either case, antibodies (of the various types to be described) can be raised against specific protein species (by the methods to be described) and their effects screened. The effects of the antibodies can be assayed and suitable antibodies selected that raise or lower the target protein species concentration and/or activity. Such assays involve introducing antibodies into a cell (see below), and assaying the concentration of the wild-type amount or activities of the target protein by standard means (such as immunoassays) known in the art. The net

activity of the wild-type form can be assayed by assay means appropriate to the known activity of the target protein.

Antibodies can be introduced into cells in numerous fashions, including, for example, microinjection of antibodies into a cell (Morgan *et al.*, 1988, *Immunology Today* 9:84-86) or transforming hybridoma mRNA encoding a desired antibody into a cell (Burke *et al.*, 1984, *Cell 36*:847-858). In a further technique, recombinant antibodies can be engineering and ectopically expressed in a wide variety of non-lymphoid cell types to bind to target proteins as well as to block target protein activities (Biocca *et al.*, 1995, *Trends in Cell Biology 5*:248-252). Preferably, expression of the antibody is under control of a controllable promoter, such as the Tet promoter. A first step is the selection of a particular monoclonal antibody with appropriate specificity to the target protein (see below). Then sequences encoding the variable regions of the selected antibody can be cloned into various engineered antibody formats, including, for example, whole antibody, Fab fragments, Fv fragments, single chain Fv fragments ($V_H$ and $V_L$ regions united by a peptide linker) ("ScFv" fragments), diabodies (two associated ScFv fragments with different specificities), and so forth (Hayden *et al.*, 1997, *Current Opinion in Immunology* 9:210-212). Intracellularly expressed antibodies of the various formats can be targeted into cellular compartments (*e.g.*, the cytoplasm, the nucleus, the mitochondria, etc.) by expressing them as fusions with the various known intracellular leader sequences (Bradbury *et al.*, 1995, *Antibody Engineering*, vol. 2, Borrebaeck ed., IRL Press, pp 295-361). In particular, the ScFv format appears to be particularly suitable for cytoplasmic targeting.

Antibody types include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. Various procedures known in the art may be used for the production of polyclonal antibodies to a target protein. For production of the antibody, various host animals can be immunized by injection with the target protein, such host animals include, but are not limited to, rabbits, mice, rats, etc. Various adjuvants can be used to increase the immunological response, depending on the host species, and include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, and potentially useful human adjuvants such as bacillus Calmette-Guerin (BCG) and corynebacterium parvum.

For preparation of monoclonal antibodies directed towards a target protein, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. Such techniques include, but are not restricted to, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256: 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology*

*Today 4*: 72), and the EBV hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985, *in Monoclonal Antibodies and Cancer Therapy,* Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A. 80*: 2026-2030), or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, 1985, in *Monoclonal Antibodies and Cancer Therapy,* Alan R. Liss, Inc., pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. U.S.A. 81*: 6851-6855; Neuberger *et al.*, 1984, *Nature 312*:604-608; Takeda *et al.*, 1985, *Nature 314*: 452-454) by splicing the genes from a mouse antibody molecule specific for the target protein together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

Additionally, where monoclonal antibodies are advantageous, they can be alternatively selected from large antibody libraries using the techniques of phage display (Marks *et al.*, 1992, *J. Biol. Chem. 267*:16007-16010). Using this technique, libraries of up to $10^{12}$ different antibodies have been expressed on the surface of fd filamentous phage, creating a "single pot" *in vitro* immune system of antibodies available for the selection of monoclonal antibodies (Griffiths *et al.*, 1994, *EMBO J. 13*:3245-3260). Selection of antibodies from such libraries can be done by techniques known in the art, including contacting the phage to immobilized target protein, selecting and cloning phage bound to the target, and subcloning the sequences encoding the antibody variable regions into an appropriate vector expressing a desired antibody format.

According to the invention, techniques described for the production of single chain antibodies (U.S. patent 4,946,778) can be adapted to produce single chain antibodies specific to the target protein. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse *et al.*, 1989, *Science 246*: 1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the target protein.

Antibody fragments that contain the idiotypes of the target protein can be generated by techniques known in the art. For example, such fragments include, but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, the Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent, and Fv fragments.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.*, ELISA (enzyme-linked immunosorbent assay). To select antibodies specific to a target protein, one may assay generated hybridomas or a phage display antibody library for an antibody that binds to the target protein.

### 5.3.5. METHODS OF MODIFYING PROTEIN ACTIVITIES

Methods of directly modifying protein activities include, *inter alia*, dominant negative mutations, specific drugs (used in the sense of this application) or chemical moieties generally, and also the use of antibodies, as previously discussed.

Dominant negative mutations are mutations to endogenous genes or mutant exogenous genes that when expressed in a cell disrupt the activity of a targeted protein species. Depending on the structure and activity of the targeted protein, general rules exist that guide the selection of an appropriate strategy for constructing dominant negative mutations that disrupt activity of that target (Hershkowitz, 1987, *Nature 329*:219-222). In the case of active monomeric forms, over expression of an inactive form can cause competition for natural substrates or ligands sufficient to significantly reduce net activity of the target protein. Such over expression can be achieved by, for example, associating a promoter, preferably a controllable or inducible promoter, of increased activity with the mutant gene. Alternatively, changes to active site residues can be made so that a virtually irreversible association occurs with the target ligand. Such can be achieved with certain tyrosine kinases by careful replacement of active site serine residues (Perlmutter *et al.*, 1996, *Current Opinion in Immunology 8*:285-290).

In the case of active multimeric forms, several strategies can guide selection of a dominant negative mutant. Multimeric activity can be controllably decreased by expression of genes coding exogenous protein fragments that bind to multimeric association domains and prevent multimer formation. Alternatively, controllable over expression of an inactive protein unit of a particular type can tie up wild-type active units in inactive multimers, and thereby decrease multimeric activity (Nocka *et al.*, 1990, *EMBO J. 9*:1805-1813). For example, in the case of dimeric DNA binding proteins, the DNA binding domain can be deleted from the DNA binding unit, or the activation domain deleted from the activation unit. Also, in this case, the DNA binding domain unit can be expressed without the domain causing association with the activation unit. Thereby, DNA binding sites are tied up without any possible activation of expression. In the case where a particular type of unit normally undergoes a conformational change during activity, expression of a rigid unit can inactivate resultant complexes. For a further example, proteins involved in cellular

mechanisms, such as cellular motility, the mitotic process, cellular architecture, and so forth, are typically composed of associations of many subunits of a few types. These structures are often highly sensitive to disruption by inclusion of a few monomeric units with structural defects. Such mutant monomers disrupt the relevant protein activities and can be controllably expressed in a cell.

In addition to dominant negative mutations, mutant target proteins that are sensitive to temperature (or other exogenous factors) can be found by mutagenesis and screening procedures that are well-known in the art.

Also, one of skill in the art will appreciate that expression of antibodies binding and inhibiting a target protein can be employed as another dominant negative strategy.

### 5.3.6. DRUGS OF SPECIFIC KNOWN ACTION

Finally, activities of certain target proteins can be controllably altered by exposure to exogenous drugs or ligands. In a preferable case, a drug is known that interacts with only one target protein in the cell and alters the activity of only that one target protein. Graded exposure of a cell to varying amounts of that drug thereby causes graded perturbations of pathways originating at that protein. The alteration can be either a decrease or an increase of activity. Less preferably, a drug is known and used that alters the activity of only a few (*e.g.*, 2-5) target proteins with separate, distinguishable, and non-overlapping effects. Graded exposure to such a drug causes graded perturbations to the several pathways originating at the target proteins.

## 6. REFERENCES CITED

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. While preferred embodiments have been described, these descriptions are merely illustrative and are not intended to limit their scope. For example, although preferred embodiments described above were in the context of a system for storage, retrieval, and analysis of biological expression array data, those skilled in the art will recognize that the disclosed methods and structures are readily adaptable for broader applications. For example, the preferred embodiments are readily applicable to a variety of tools that analyzing large amounts of machine-readable biological experiment data. Further, one skilled in the art will note that,

although the concept of "hyperbolic trees" is discussed, hyperbolic trees are by no means the only form of cluster visualization utilized by the methods of the present invention. The concept of a hyperbolic tree is only one embodiment of the cluster visualization envisioned by the patent and serves as a non-limiting example. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

What is claimed is:

1. A computer program product for directing a user computer in a computer-aided analysis of biological response data, the biological response data being derived from machine readable outputs of biological signal experiments performed on one or more biological samples, said computer program product comprising:

computer code for receiving search commands from a user and displaying information related to said biological response data on a plurality of viewers on a user display;

computer code for receiving a source dataset selection command from the user for identifying a source dataset, said source dataset corresponding to a subset of biological data being displayed on a source viewer, said source viewer being one of said plurality of viewers;

computer code for projecting said source dataset onto a destination viewer, said projection being characterized by a highlighted display of data in said destination viewer that corresponds to said source dataset from said source viewer, said destination viewer being a different one of said plurality of viewers than said source viewer;

whereby user observation of biological relationships that may exist in said biological samples may be enhanced through the facilitated viewing of said source dataset as projected onto said destination viewer.

2. The computer program product of claim 1, said computer code for receiving search commands comprising computer code for displaying a customizable search menu and computer code for receiving menu and alphanumeric inputs from the user and automatically building database queries therefrom.

3. The computer program product of claim 2, said computer code for receiving a source dataset selection command comprising computer code for displaying a search menu and computer code for receiving menu and alphanumeric inputs from the user, wherein said computer code for receiving a source dataset selection command enables a look and feel similar to that of said computer code for receiving search commands.

4. The computer program product of claim 2, said computer code for receiving a source dataset selection command comprising computer code that allows the user to graphically select the source dataset directly from the source viewer.

5. The computer program product of claim 4, said graphical selection being performed with a mouse lasso technique.

6. The computer program product of claim 5, wherein each of said biological response viewers is capable of being prevented from displaying projected datasets if the user invokes a disable projections command.

7. The computer program product of claim 6, wherein said highlighting of information corresponding to said source dataset in said destination viewer is achieved using different colors, intensities, or contrasts for said source dataset information as compared to other colors used for displaying non-projected data points.

8. The computer program product of claim 7, wherein said biological data corresponds to expression array data, and wherein said plurality of viewers are selected from a set of viewers comprising the following: an expression image viewer, a signature plot viewer, a correlation plot viewer, a trend plot viewer, and a cluster tree viewer.

9. The computer program product of claim 8, wherein said source viewer corresponds to said cluster tree viewer, said cluster tree viewer being adapted for hyperbolic display of a set of biological signal profiles.

10. The computer program product of claim 8, wherein said source viewer corresponds to said signature plot viewer, wherein said source dataset comprises genes identified as points on said signature plot viewer, and wherein the user performs graphical selection of said genes using a mouse lasso technique, whereby genes having signature plot locations of interest may quickly be selected by the user and projected onto other viewers.

11. The computer program product of claim 1, said computer program product further comprising computer code for providing a combine feature wherein a plurality of constitutive biological signal response profiles are merged together to form said biological response data.

12. The computer program product of claim 11, wherein said plurality of constitutive biological signal response profiles are merged together to form said biological response data by averaging biological signal response data that is common to two or more constitutive biological signal profiles in said plurality of biological signal response profiles.

13. The computer program product of claim 11, wherein said plurality of constitutive biological signal response profiles are stored in a central biological database server.

14. The computer program product of claim 1, said computer program product further comprising computer code for providing a resolve feature wherein a first set of biological signal response profiles, which are selected from a plurality of biological signal response profiles, is subtracted from a second set of biological signal response profiles, which are selected from a plurality of signal response profiles.

15. The computer program product of claim 14, wherein said first biological signal response profile is measured using a first aliquot of a nominal biological sample and said second biological signal response profile is measured using a second aliquot of said nominal biological sample wherein said second aliquot of said nominal biological sample has been exposed to an amount of a test pharmacological agent prior to determination of said second biological signal response profile.

16. The computer program product of claim 1, said computer program product further comprising:

computer code for deriving gene signal profiles from said biological signal experiments; and

computer code for receiving search commands from a user and displaying information related to said gene signal profiles on a plurality of biological response viewers on a user display.

17. The computer program product of claim 16, wherein said computer program product further comprises a compare feature for comparing one or more gene signal profiles.

18. A computer program product for directing a user computer in a computer-aided analysis of biological signals, the expression array data being derived from machine readable outputs of biological response experiments performed on a plurality of biological samples, said computer program product comprising:

computer code for generating a cluster tree using said expression array data; and

computer code for displaying said cluster tree in a hyperbolic display format.

19. The computer program product of claim 18, wherein said computer code for displaying said cluster tree comprises computer code for receiving scaling and magnification commands from a user for adjusting the scaling and magnification of said cluster tree display.

20. The computer program product of claim 19, said computer code for displaying said cluster tree comprising computer code for allowing the user to rotate said cluster tree about a location thereon based on user input.

21. The computer program product of claim 19, said computer code for displaying said cluster tree comprising computer code for isolating and expanding a subtree of said cluster tree.

22. The computer program product of claim 18, further comprising computer code for locating a common parent node for a set of biological response profiles selected on a cluster tree, said biological response profiles corresponding to genes, gene products, or biological experiments.

23. A method for processing biological signal profile data for storage in a central biological database server and for retrieval therefrom by a user computer connected to the central database server across a computer network, comprising the steps of:

storing a plurality of biological signal profiles in said central database server;

computing a correlation metric between at least one of said plurality of biological signal profiles and other of said plurality of biological signal profiles in said central database server;

storing said correlation metrics in said central database server in conjunction with said expression array experiments;

at said user computer, at a time subsequent to said step of storing said correlation metrics, selecting a query profile and a target set of biological response profiles from among said plurality of biological response profiles in said central database server;

receiving correlation criteria and a correlation execution command from a user at said user computer;

querying said central database server to identify biological response profiles between said source set and said target set having correlation metrics that satisfy said correlation criteria;

transmitting information related to said identified biological response profiles and related correlation metrics from said cental biological database server to said user computer;

and

displaying information derived from said biological response profiles and related correlation metrics at said user computer;

whereby real time computation of said correlation metrics is not required at said user computer after said correlation execution command, thereby enhancing speed of biological correlation analysis at said user computer.

24. The method of claim 23, said step of selecting a query profile and a target set of biological signal profiles comprising the steps of displaying a search menu and receiving menu and alphanumeric inputs from the user.

25. The method of claim 23, said step of selecting a query profile and a target set of biological signal profiles comprising the step of receiving graphical selection commands from the user.

26. The method of claim 23, said correlation metrics each consisting of a single scalar correlation coefficient.

27. The method of claim 23, said correlation metrics each comprising a multidimensional correlation matrix.

28. A computer program product for directing a user computer to perform dynamic menu generation for receiving a required biological data field selection from a user, the user computer connected to a central database server across a computer network, said computer program product comprising:

computer code for querying said central database server upon instantiation of said computer program product on said user computer, said query being adapted to request dynamic menu information from said central database server, said dynamic menu information defining biological data field items, subcategory items, and submenuing relationships among said biological data field items and subcategory items;

computer code for displaying a first menu on a user display, said first menu comprising at least a portion of said biological data field items and subcategory items;

computer code for receiving a first menu selection from the user;

computer code for determining whether said first menu selection is a biological data field or a subcategory item;

computer code for identifying said first menu selection as the required biological data field selection if said first menu selection is a biological data field; and

computer code for identifying submenu information contained in said dynamic menu information if said first menu selection is a subcategory item, and for displaying said submenu information on a second menu near said first menu on said user display;

whereby said user computer is not required to store dynamic menu information, said central database server being capable of storing substantially all required dynamic menu information, allowing for enhanced flexibility and customization capability of biological menu systems on said user computer.

29. The computer program product of claim 28, further comprising:

computer code for receiving a second menu selection from the user;

computer code for determining whether said second menu selection is a biological data field or a subcategory item;

computer code for identifying said second menu selection as the required biological data field selection if said second menu selection is a biological data field; and

computer code for identifying further submenu information contained in said dynamic menu information if said second menu selection is a subcategory item, and for displaying said further submenu information on a third menu near said first menu on said user display.

30. The computer program product of claim 29, wherein said first, second, and third menus are displayed in a pull-down format, wherein a top item of said second menu appears parallel to said selected subcategory item of the first menu, and wherein a top item of said third menu appears parallel to said selected subcategory item of the second menu, whereby clarified presentation of said menus is achieved.

31. A method for analyzing expression array data using a computer, the expression array data being derived from machine readable outputs of perturbation experiments performed on a plurality of biological samples, comprising the steps of:

generating a coregulation tree using said expression array data;

displaying said coregulation tree in a hyperbolic display format;

manipulating the display of the coregulation tree to display a first gene or gene product located on a branch thereof, said first gene or gene product having a known function;

locating a second gene or gene product on said coregulation tree on the same branch as said first gene or gene product;

and

assigning a function to said second gene or gene product using information related to said known function of said first gene or gene product and information related to a positional relationship or distance metric between said second gene or gene product and said first gene or gene product on said branch of said coregulation tree.

32. A computer program product for directing a user computer in a computer-aided analysis of expression array data, the expression array data comprising an image of an expression array, comprising:

computer code for computing one or more quality statistics relating to the image;

and

computer code for simultaneously displaying on a computer screen said quality statistics alongside said image.

33. The computer program product of claim 32, further comprising:

computer code for receiving a user command selecting a spot on said image display corresponding to a particular gene or gene product;

computer code for enlarging said spot;

and

computer code for simultaneously displaying on said computer screen said enlarged spot alongside said image and said quality statistics.

34. A method for analyzing expression array data using a computer, the expression array data being derived from machine readable outputs of perturbation experiments performed on a plurality of biological samples, comprising the steps of:

generating an experiment profile cluster tree using said expression array data;

displaying said experiment profile cluster tree in a hyperbolic display format;

manipulating the display of the experiment profile cluster tree to display a first experiment located on a branch thereof, said first experiment corresponding to a known function of a perturbation assayed therein;

locating a second experiment on said experiment profile cluster tree on the same branch as said first experiment;

and

assigning a function to a biological perturbation assayed in said second experiment using information related to said known function of said perturbation assayed in said first experiment and information related to a positional relationship or distance metric between said second experiment and said first experiment on said branch of said experiment profile cluster tree.

35. A method of validating a drug target, said method comprising using a computer program product according to Claim 14, wherein a first set of biological signal response profiles, which are selected from a plurality of biological signal response profiles, is subtracted from a second set of biological signal response profiles, which are selected from a plurality of signal response profiles.

36. A computer program product for use in conjunction with a computer system, the computer program product comprising a computer readable storage medium and a computer program mechanism embedded therein, the computer program mechanism comprising:

instructions for displaying biological data on a user display;

instructions for receiving a selection of a subset of said biological data from a user;

instructions for generating a context-sensitive menu in response to said selection, wherein a menu option in said context-sensitive menu comprises a substitution tag;

instructions for obtaining a first menu selection from said user;

instructions for replacing a substitution tag in said first menu selection, said replacing being based on an operation that uses a portion of said subset of said biological data, thereby building a substituted menu option; and

instructions for executing said substituted menu option.

37. The computer program product of claim 36 wherein said menu option is selected from the group consisting of a command and a link.

38. The computer program product of claim 37 wherein said menu option is a link and said substitution tag is selected from the group consisting of a value substitution tag, a structured query language substitution tag, and a repeat substitution tag.

39. The computer program product of claim 36 wherein said substitution tag is nestable.

40. The computer program product of claim 38 wherein said substitution tag is a structured query language substitution tag that begins with a select operation.

41. The computer program product of claim 36 wherein said instructions for generating a context-sensitive menu in response to said selection further comprises:

instructions for accessing a machine-readable file that comprises said menu option; and

instructions for building said context-sensitive menu using said menu option in said machine-readable file.

42. The computer program product of claim 41 wherein said machine-readable file is located on a centralized server and said instructions for accessing said machine-readable file comprises instructions for accessing said machine-readable file across a network.

43. The computer program product of claim 36 wherein said menu option in said context-sensitive menu may be edited by a system administrator without recompiling said computer program product.

44. The computer program product of claim 36 wherein said instructions for receiving a selection of a subset of said biological data from a user comprises a right-click event that is initiated by said user.

45. A method for generating a context-sensitive menu for use in building and executing a customized menu option that is related to the study of biological information, said method comprising the steps of:

displaying biological data on a user display;

receiving a selection of a subset of said biological data from a user;

generating a context-sensitive menu in response to said selection of said subset of said biological data, wherein a menu option in said context-sensitive menu comprises a substitution tag;

obtaining a first menu selection from said user;

replacing a substitution tag in said menu selection choice, said replacing being based on an operation that uses a portion of said subset of said biological data, thereby building a substituted menu option; and

executing said substituted menu option; wherein said executed substituted menu option provides useful biological information that is related to said subset of said biological data selected by said user.

46. The method of claim 45 wherein said menu option is selected from the group consisting of a command and a link.

47. The method of claim 45 wherein said menu option is a link and said substitution tag is selected from the group consisting of a value substitution tag, a structured query language substitution tag, and a repeat substitution tag.

48. The method of claim 45 wherein said substitution tag is nestable.

49. The method of claim 47 wherein said substitution tag is a structured query language substitution tag that begins with a select operation.

50. The method of claim 45 wherein said step of generating a context-sensitive menu in response to said selection further comprises the steps of:

accessing a machine-readable file that comprises said menu option; and

building said context-sensitive menu using said menu option in said machine-readable file.

51. The method of claim 50 wherein said machine-readable file is located on a centralized server and said accessing step comprises the step of establishing a communication link with said centralized server across a network.

52. The method of claim 45 further comprising the step of:

granting a system administrator the privilege to edit menu options in said context-sensitive menu.

53. The method of claim 45 wherein said step of receiving a selection of a subset of said biological data from a user is triggered when said user right-clicks on said biological data with an input device.

54. A computer readable memory used to direct a computer to function in a specified manner, said memory comprising a module capable of analyzing biological data; wherein said module comprises:

instructions for displaying biological data on a user display;

instructions for receiving a selection of a subset of said biological data from a user;

instructions for generating a context-sensitive menu in response to said selection, wherein a menu option in said context-sensitive menu comprises a substitution tag;

instructions for obtaining a first menu selection from said user;

instructions for replacing a substitution tag in said first menu selection, said replacing being based on an operation that uses a portion of said subset of said biological data, thereby building a substituted menu option; and

instructions for executing said substituted menu option.

55. The memory of claim 54 wherein said menu option is selected from the group consisting of a command and a link.

56. The memory of claim 55 wherein said menu option is a link and said substitution tag is selected from the group consisting of a value substitution tag, a structured query language substitution tag, and a repeat substitution tag.

57. The memory of claim 54 wherein said substitution tag is nestable.

58. The memory of claim 56 wherein said substitution tag is a structured query language substitution tag that begins with a select operation.

59. The memory of claim 54 wherein said instructions for generating a context-sensitive menu in response to said selection further comprises:

instructions for accessing a machine-readable file that comprises said menu option; and

instructions for building said context-sensitive menu using said menu option in said machine-readable file.

60. The memory of claim 59 wherein said machine-readable file is located on a centralized server and said instructions for accessing said machine-readable file comprises instructions for accessing said machine-readable file across a network.

61. The memory of claim 54 wherein said menu option in said context-sensitive menu may be edited by a system administrator without recompiling said computer program product.

62. The memory of claim 54 wherein said instructions for receiving a selection of a subset of said biological data from a user comprises a right-click event that is initiated by said user.

63. The method of claim 23 wherein said selecting of a query profile and said target set of biological response profiles is facilitated using a search tool wizard.

64. The method of claim 63 wherein said search tool wizard is selected from the group consisting of a gene search tool wizard and an experiment search tool wizard.

65. The computer program product of claim 1 wherein said computer code for receiving search commands and said computer code for receiving a source dataset selection command for identifying said source dataset is facilitated by the use of a search tool wizard.

66. The computer program product of claim 65 wherein said search tool wizard is selected from the group consisting of a gene search tool wizard and an experiment search tool wizard.

67. A computer readable memory used to direct a computer to function in a specified manner, said memory comprising a module capable of analyzing biological data; wherein said module comprises:

instructions for receiving search commands from a user and displaying information related to said biological response data on a plurality of viewers on a user display;

instructions for receiving a source dataset selection command from the user for identifying a source dataset, said source dataset corresponding to a subset of biological data being displayed on a source viewer, said source viewer being one of said plurality of viewers; and

instructions for projecting said source dataset onto a destination viewer, said projection being characterized by a highlighted display of data in said destination viewer that corresponds to said source dataset from said source viewer, said destination viewer being a different one of said plurality of viewers than said source viewer;

whereby user observation of biological relationships that may exist in said biological samples may be enhanced through the facilitated viewing of said source dataset as projected onto said destination viewer.

68. The memory of claim 67, said instructions for receiving search commands further comprising:

instructions for displaying a customizable search menu; and

instructions for receiving menu and alphanumeric inputs from the user and automatically building database queries therefrom.

69. The memory of claim 67, said module further comprising instructions for providing a combine feature wherein a plurality of constitutive biological signal response profiles are merged together to form said biological response data.

70. The memory of claim 69, wherein said plurality of constitutive biological signal response profiles are merged together to form said biological response data by averaging biological signal response data that is common to two or more constitutive biological signal profiles in said plurality of biological signal response profiles.

71. The memory of claim 69, wherein said plurality of constitutive biological signal response profiles are stored in a central biological database server.

72. The memory of claim 67, said module further comprising instructions for providing a resolve feature wherein a first set of biological signal response profiles, which are selected from a plurality of biological signal response profiles, is subtracted from a second set of biological signal response profiles, which are selected from a plurality of signal response profiles.

73. The memory of claim 72, wherein said first biological signal response profile is measured using a first aliquot of a nominal biological sample and said second biological

signal response profile is measured using a second aliquot of said nominal biological sample wherein said second aliquot of said nominal biological sample has been exposed to an amount of a test pharmacological agent prior to determination of said second biological signal response profile.

74. The memory of claim 67, said module further comprising:

instructions for deriving gene signal profiles from said biological signal experiments; and

instructions for receiving search commands from a user and displaying information related to said gene signal profiles on a plurality of biological response viewers on a user display.

75. The memory of claim 74, wherein said module further comprises a compare feature for comparing one or more gene signal profiles.

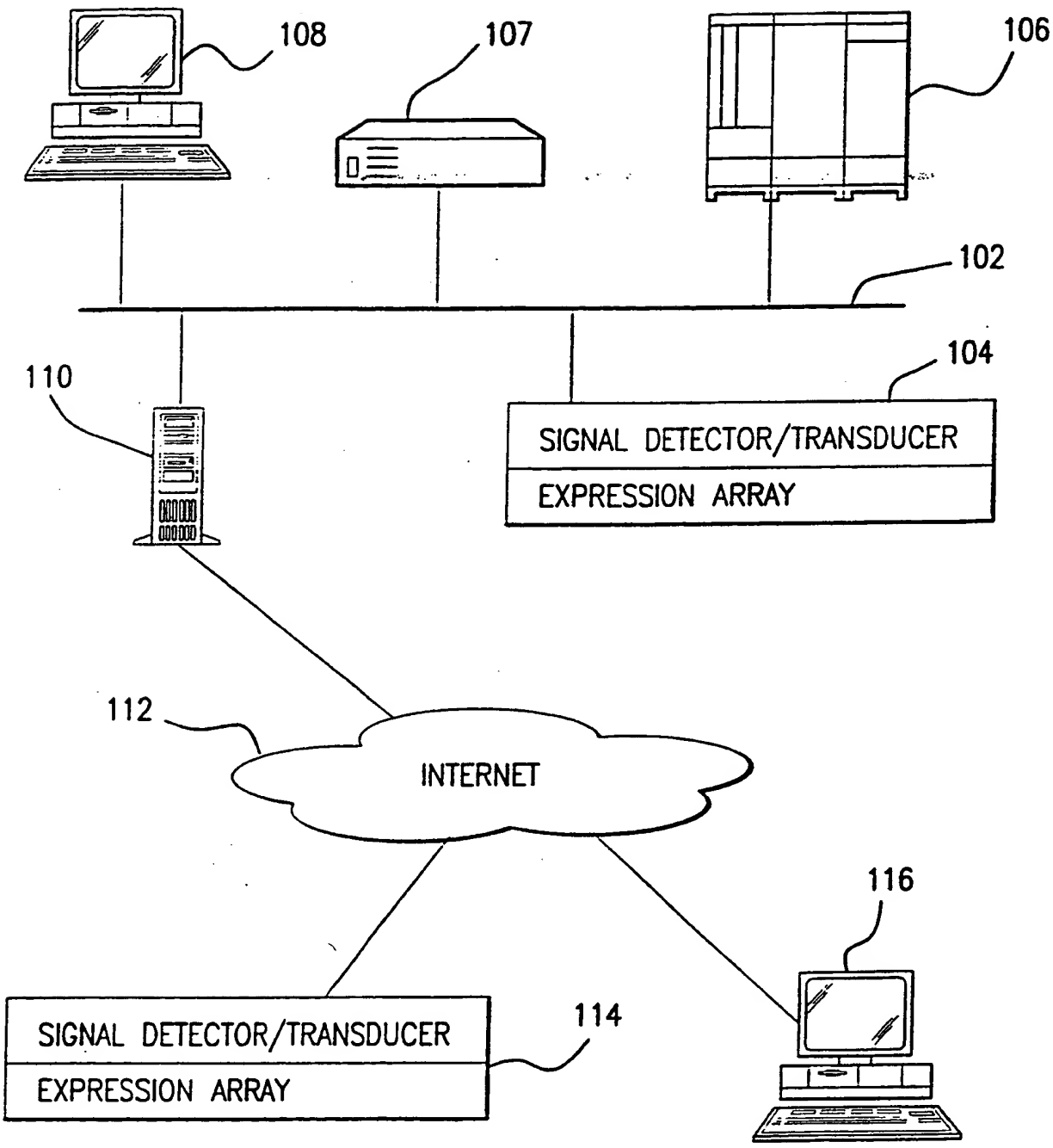
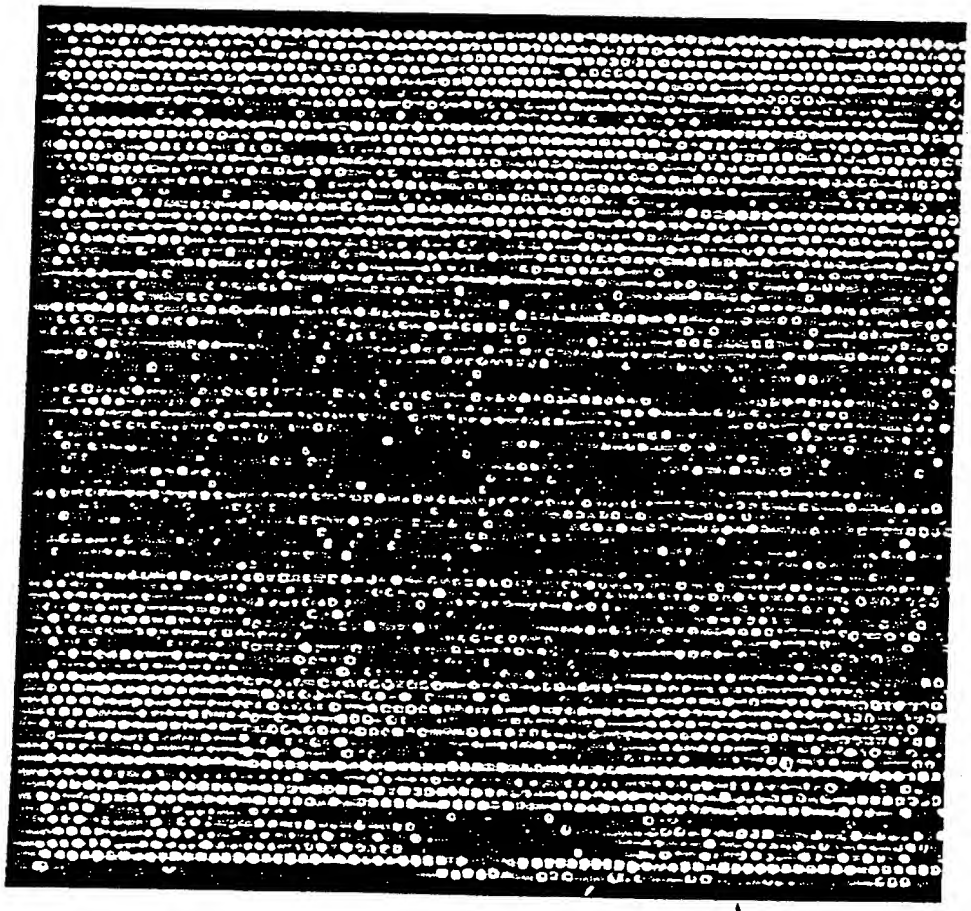



FIG.1



(PRIOR ART)

200

FIG.2

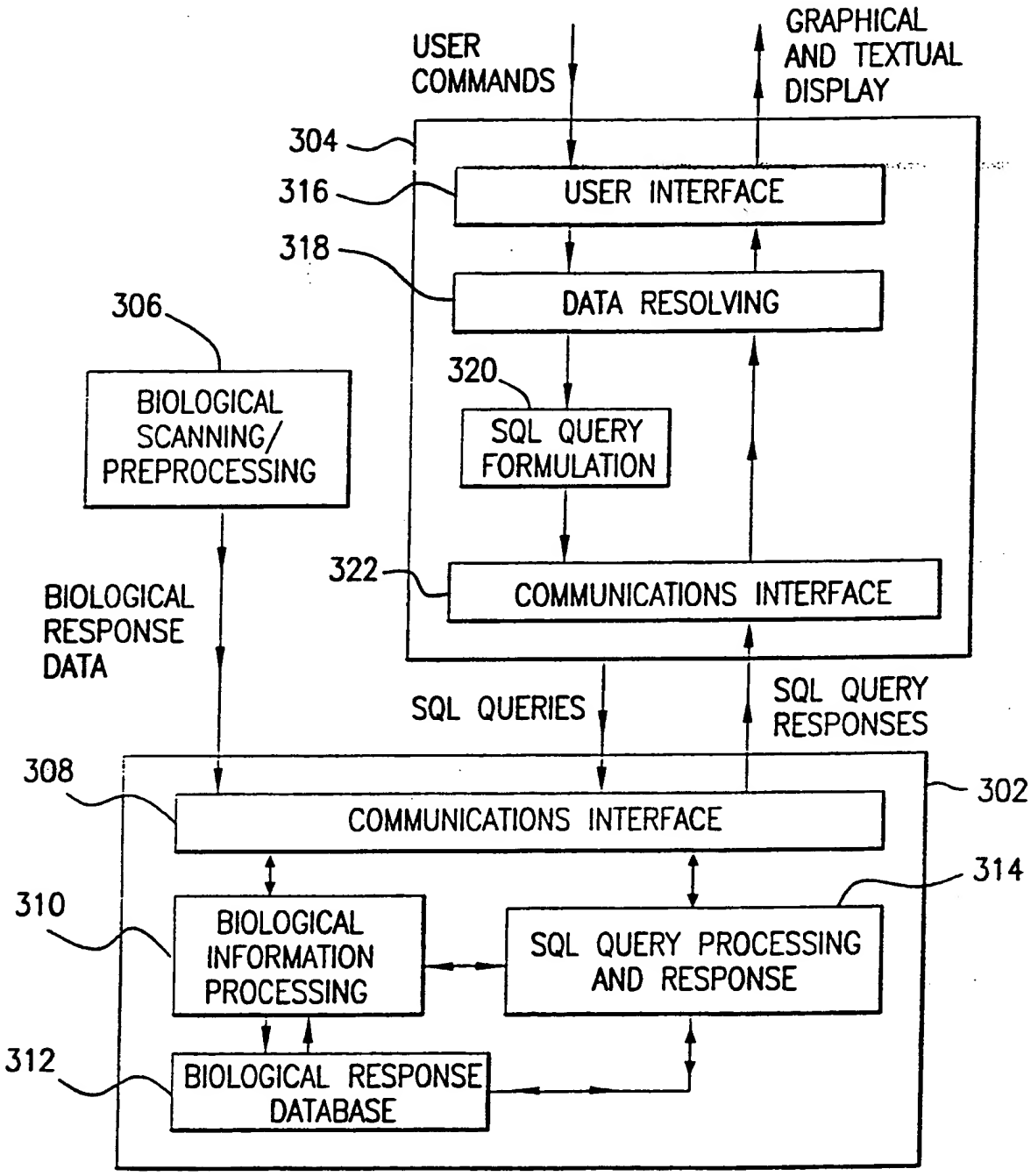



FIG.3

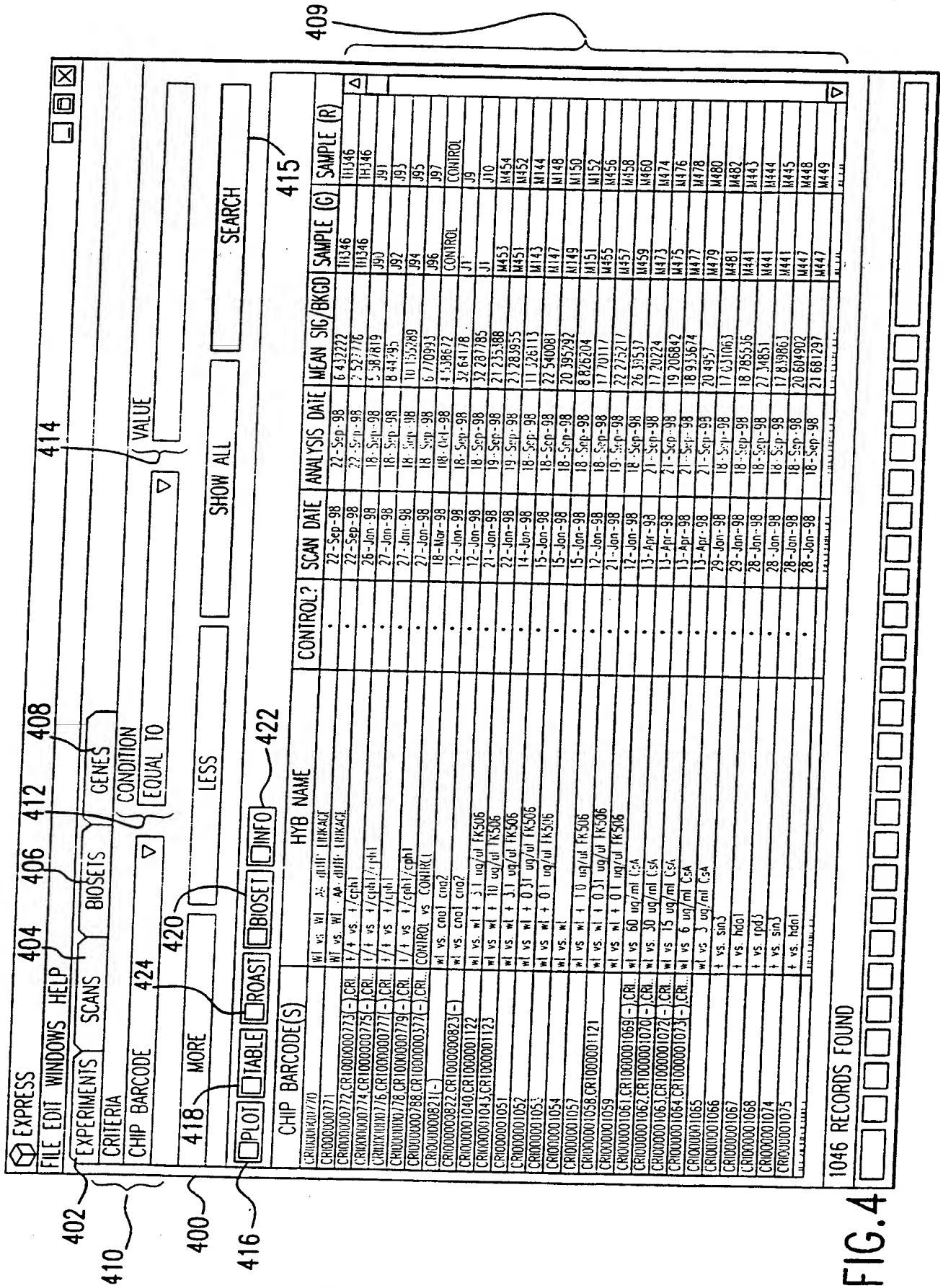



FIG. 4

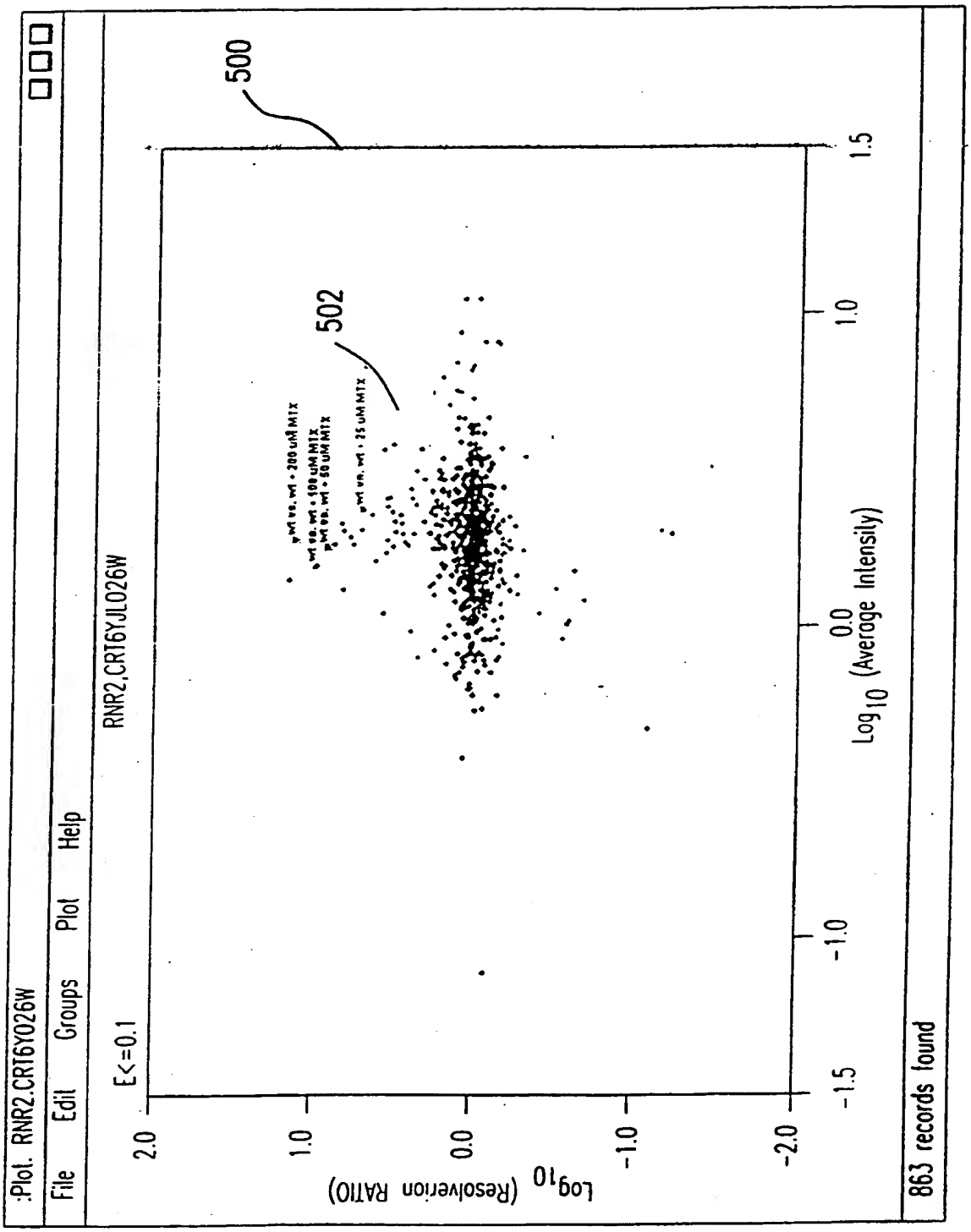



FIG.5

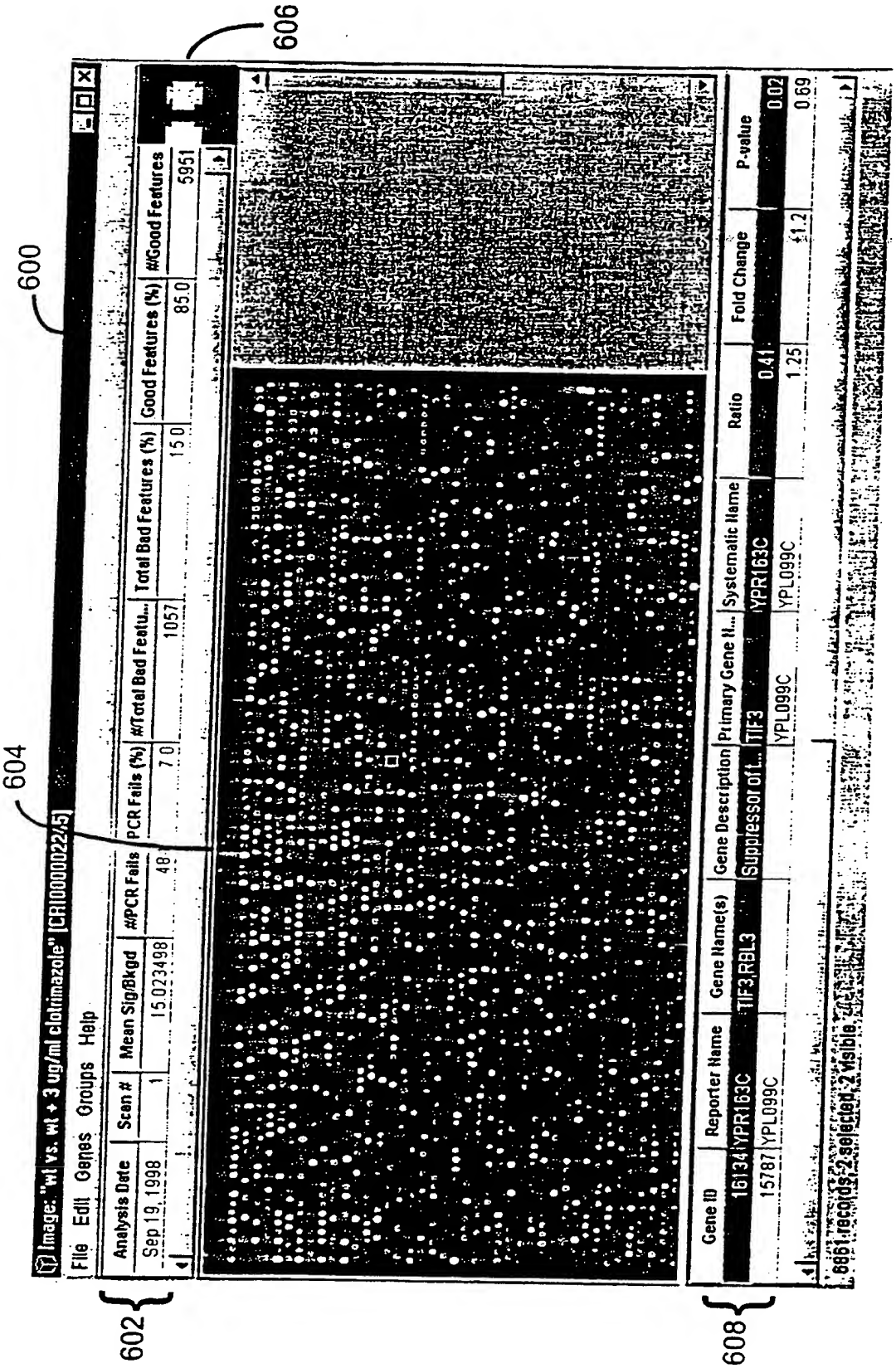



FIG.6

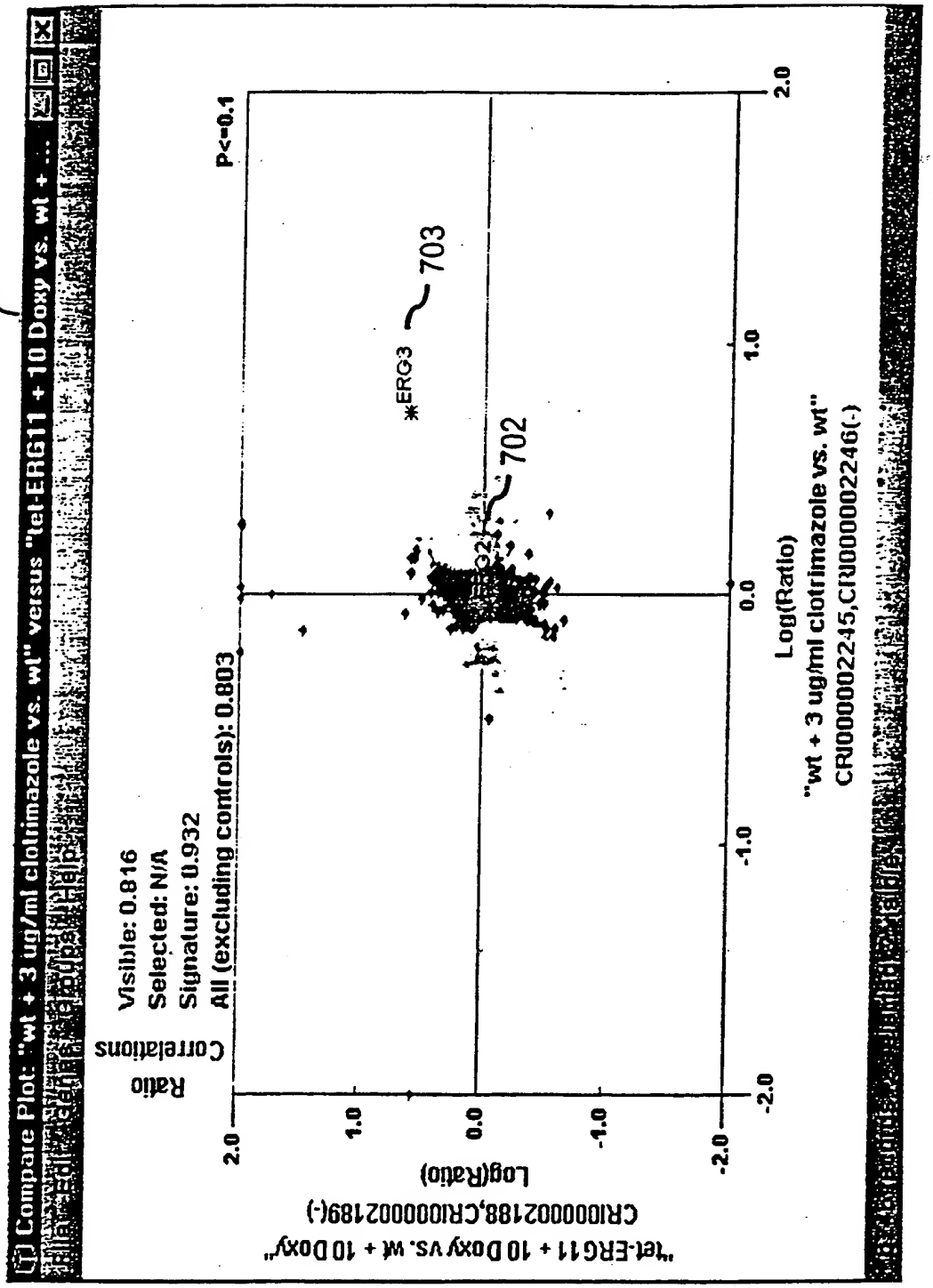
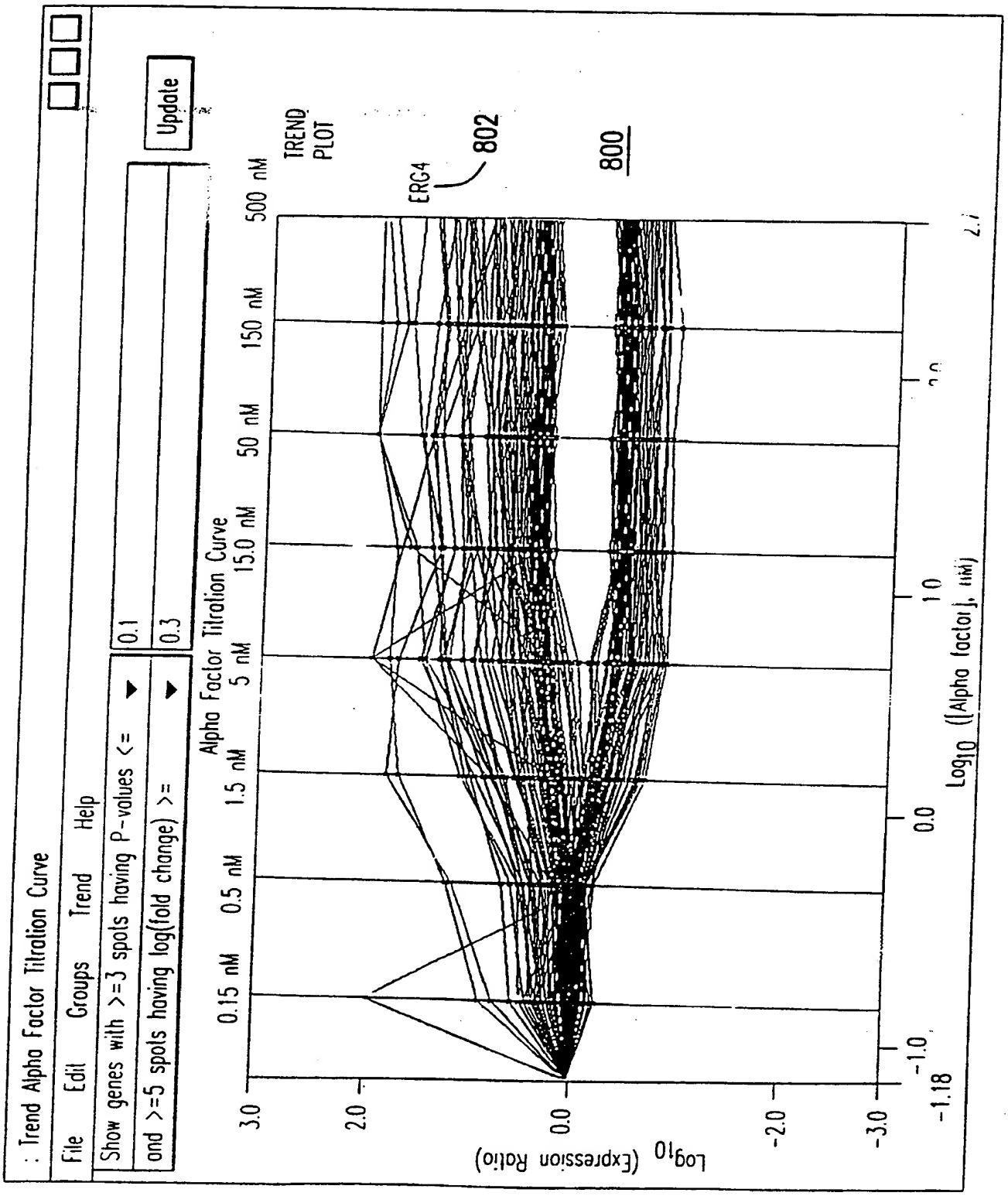



FIG.7

FIG.8



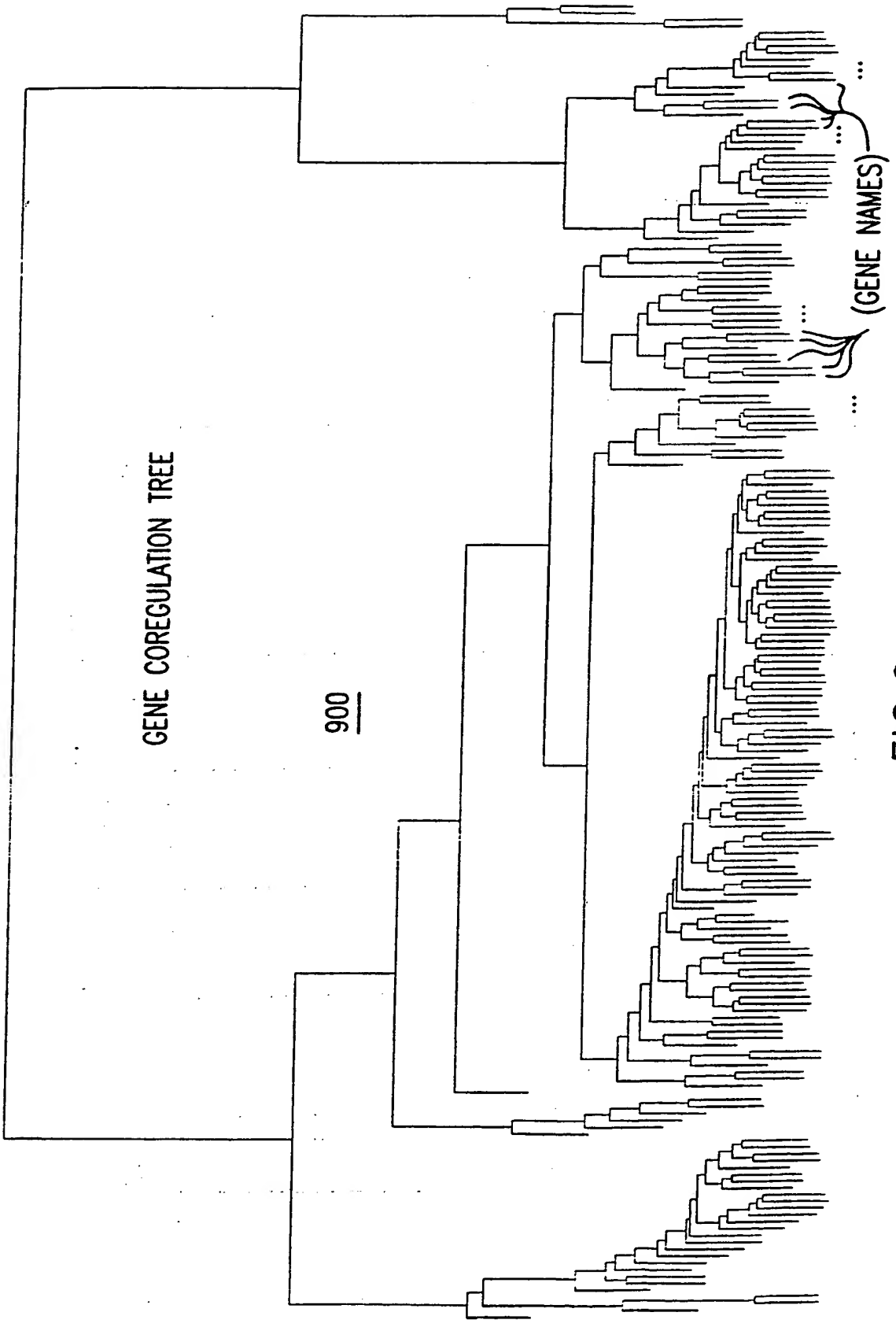



FIG.9
PRIOR ART

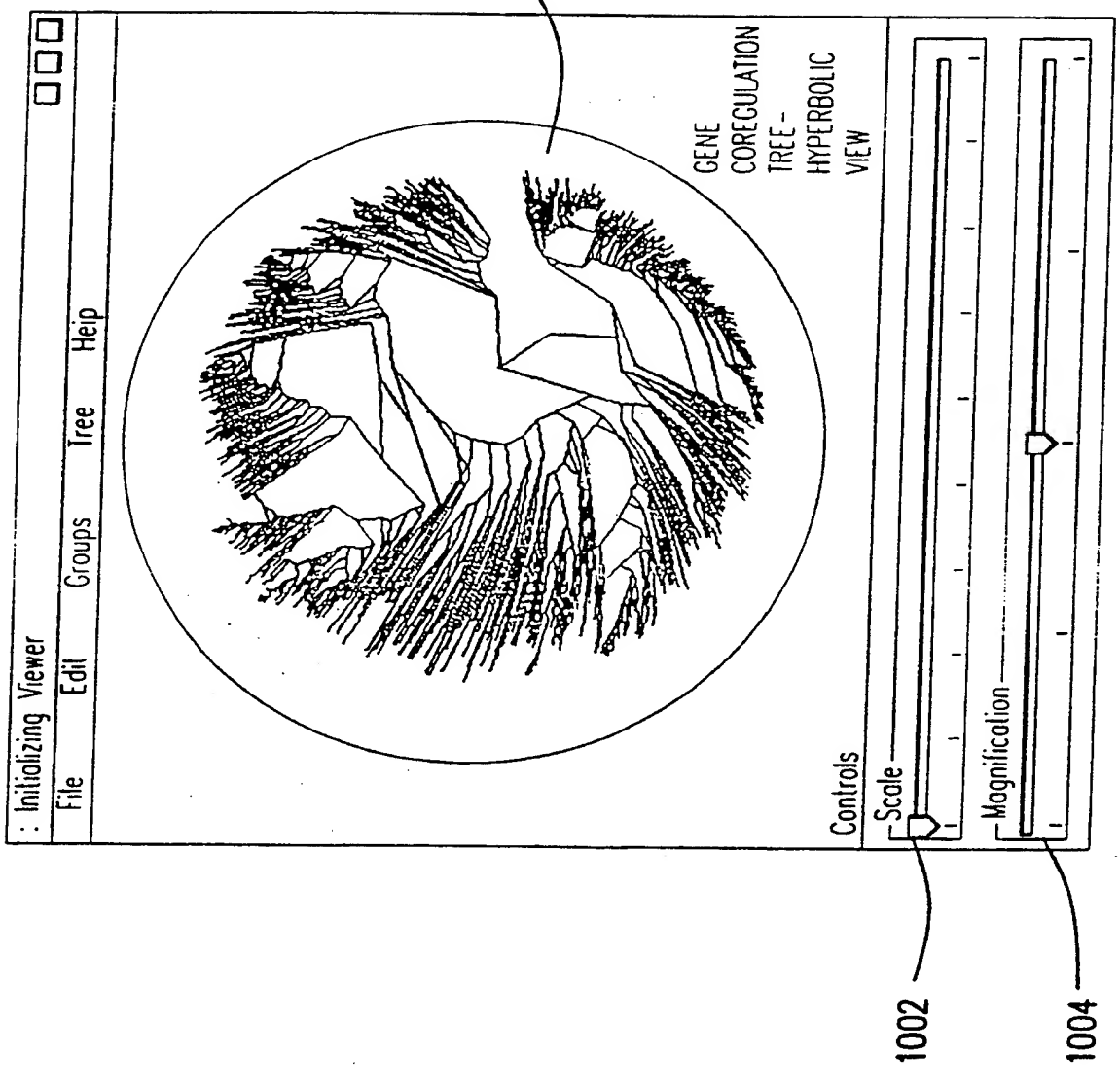



FIG.10

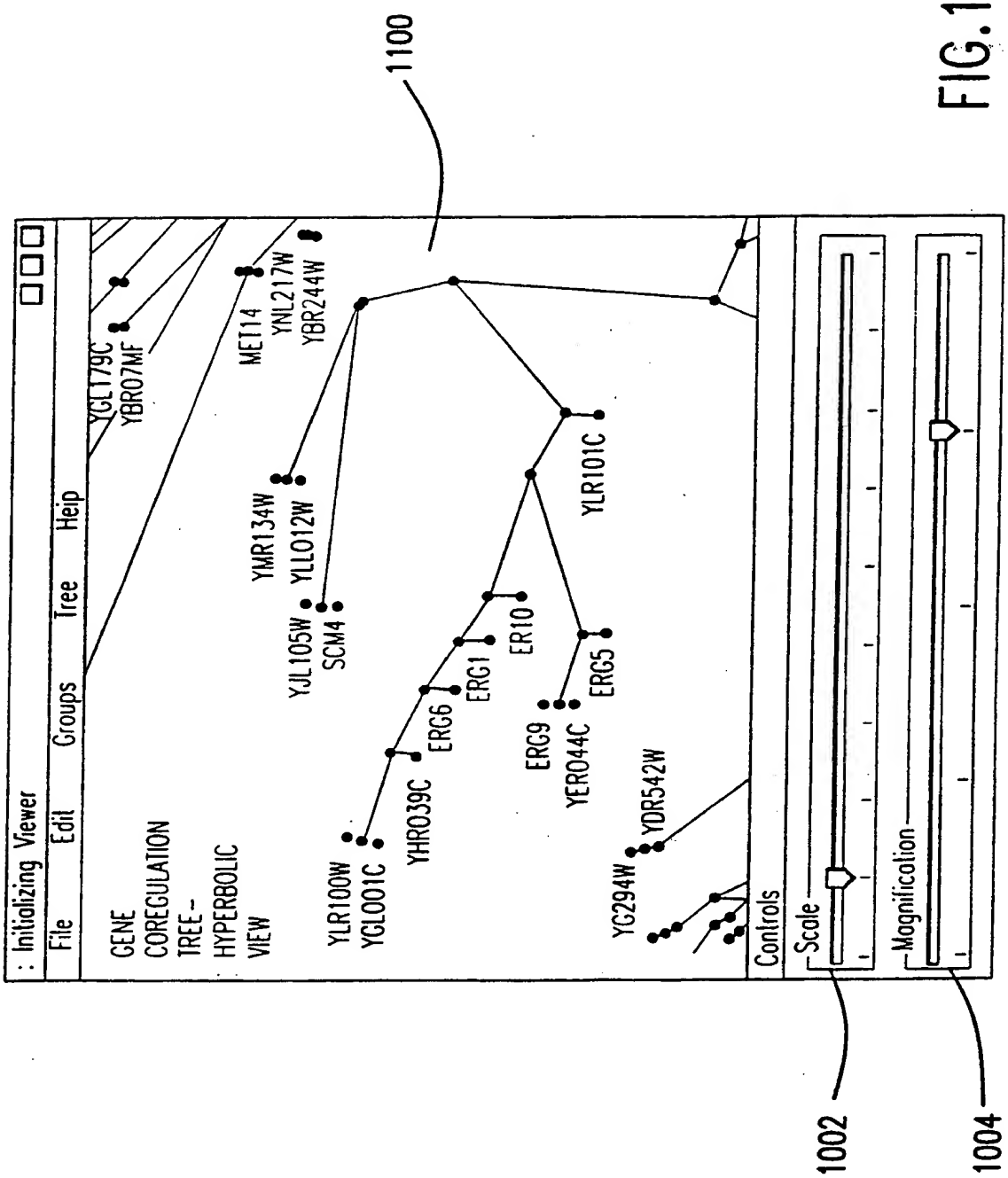



FIG.11

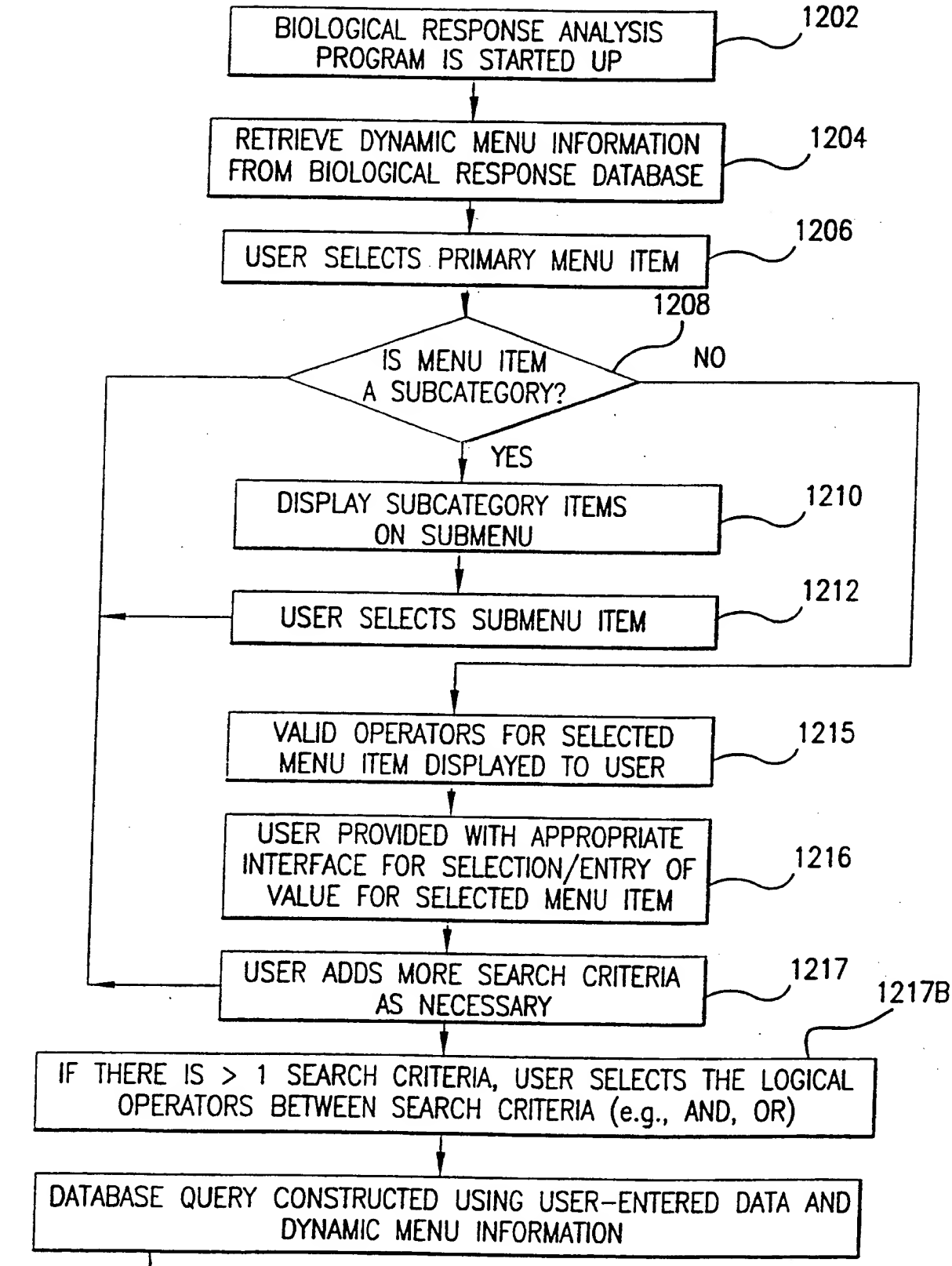



FIG.12

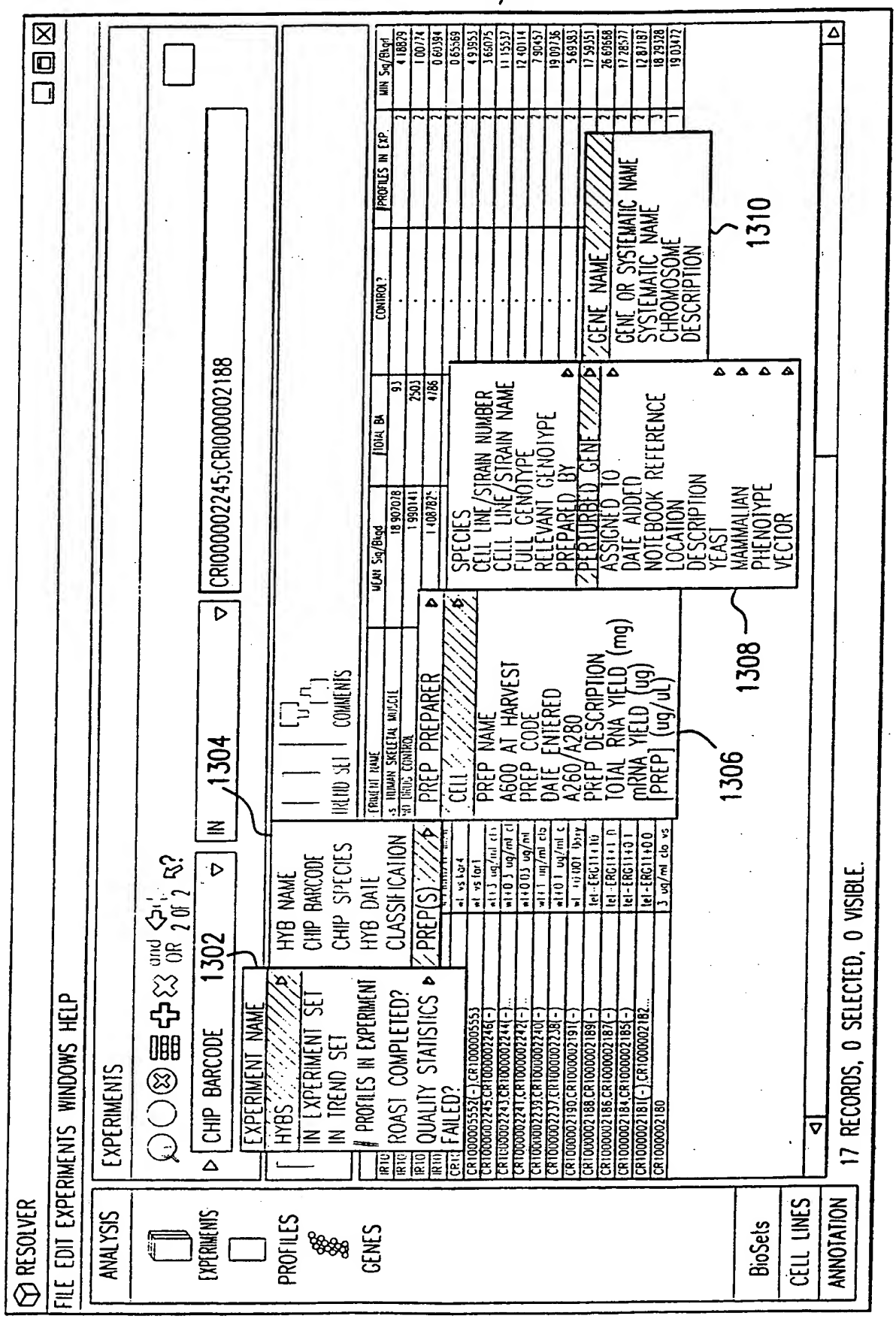



FIG.13

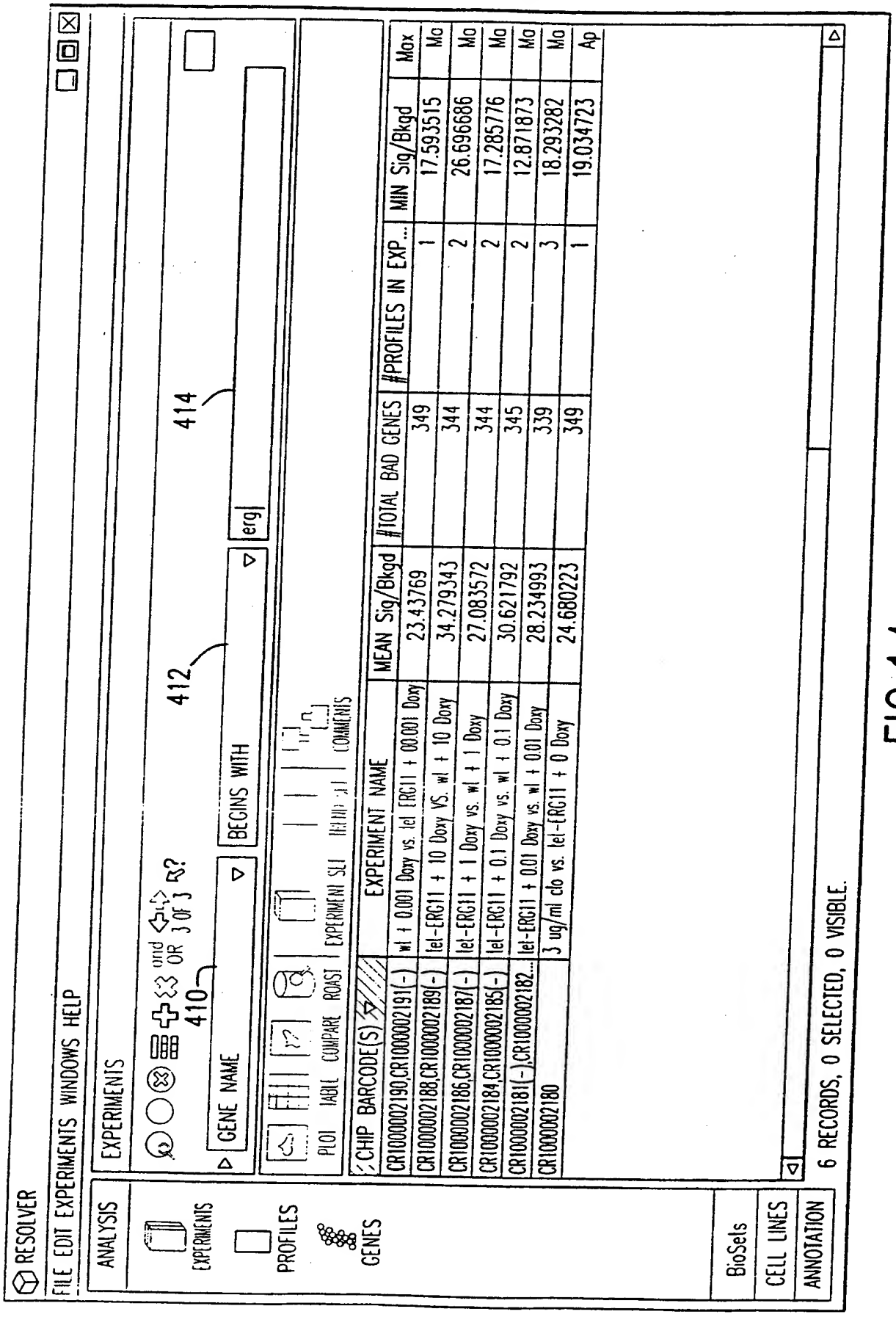



FIG.14

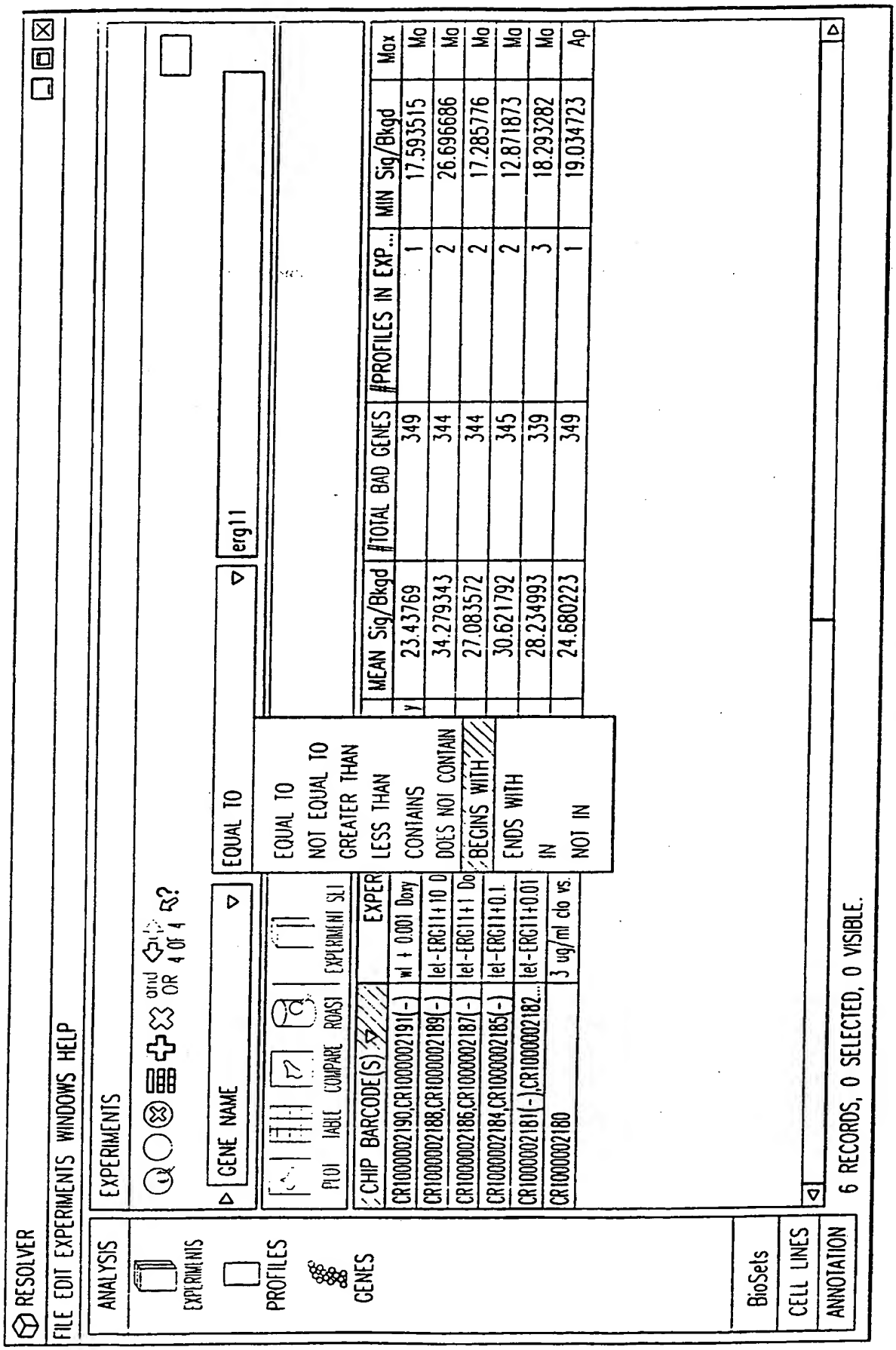



FIG.15

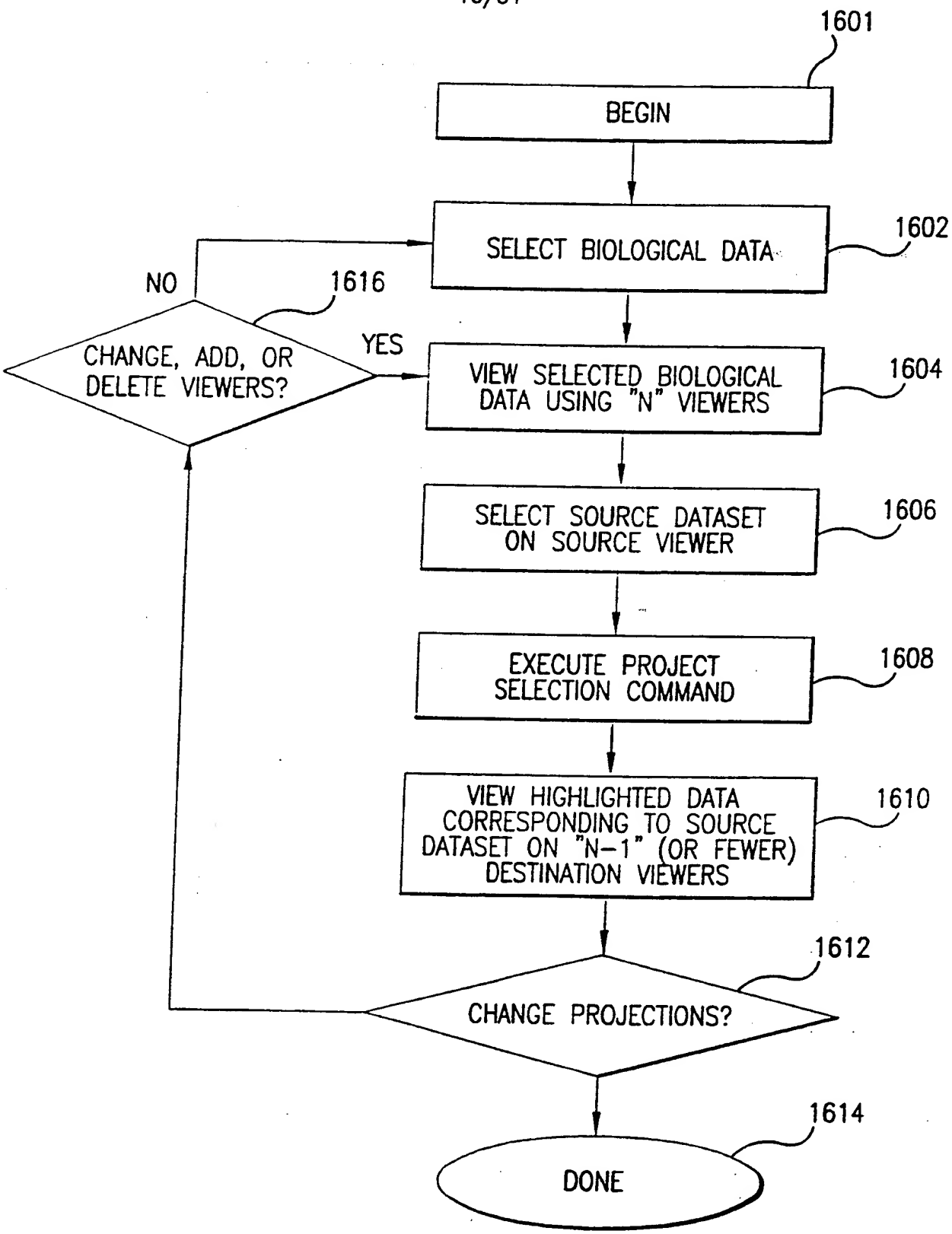



FIG. 16

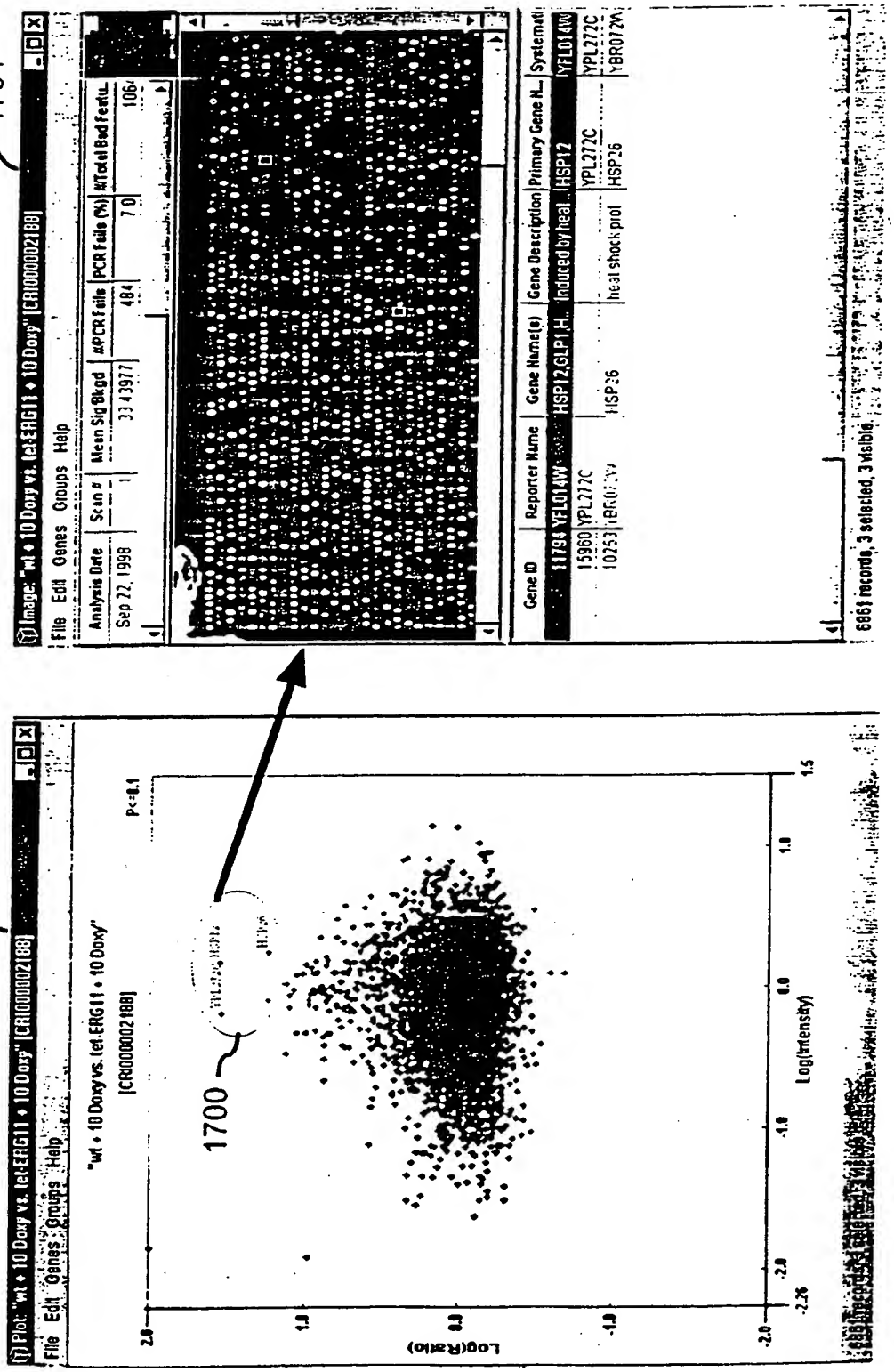



FIG.17

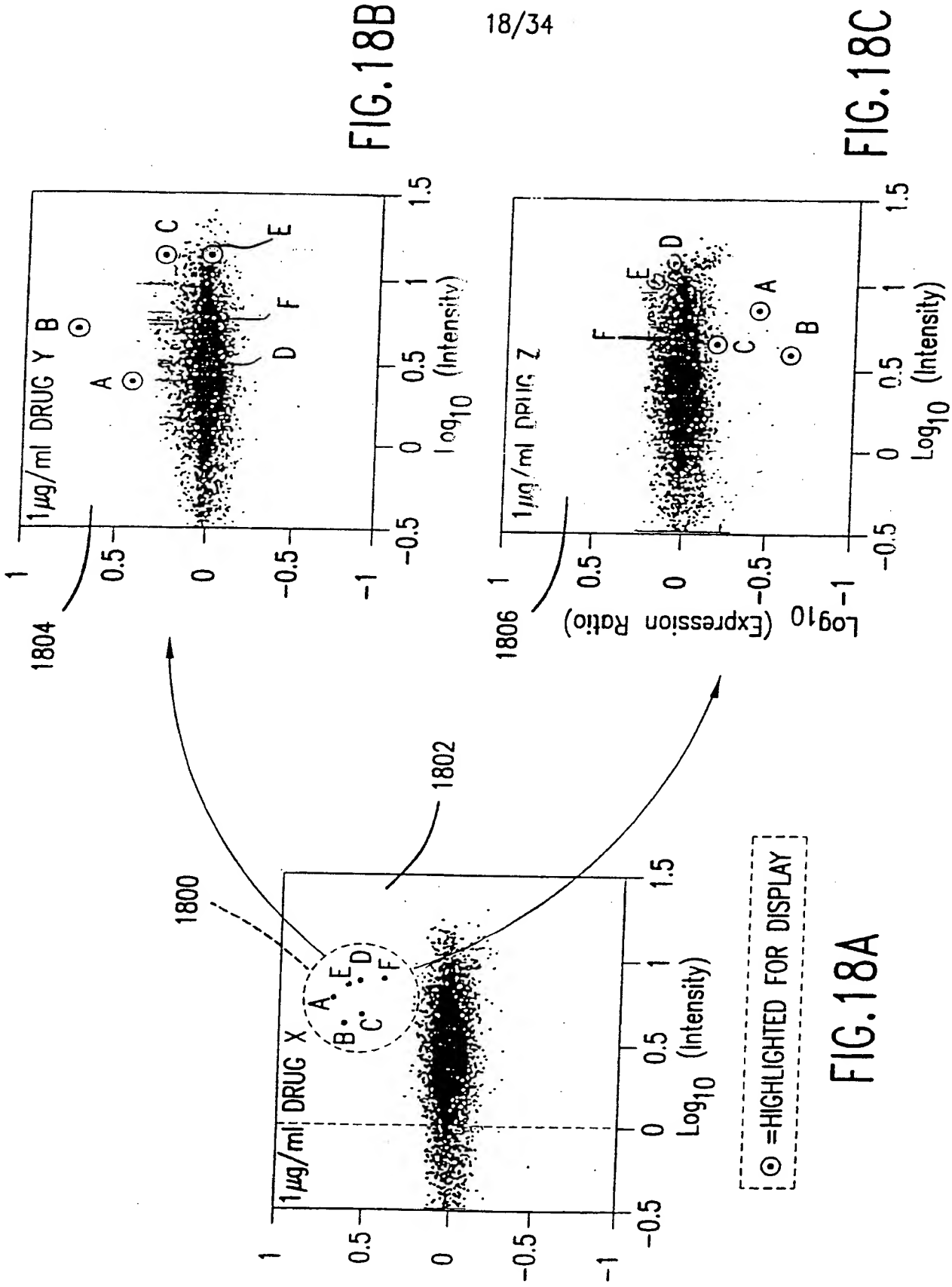



FIG.18A

FIG.18B

FIG.18C

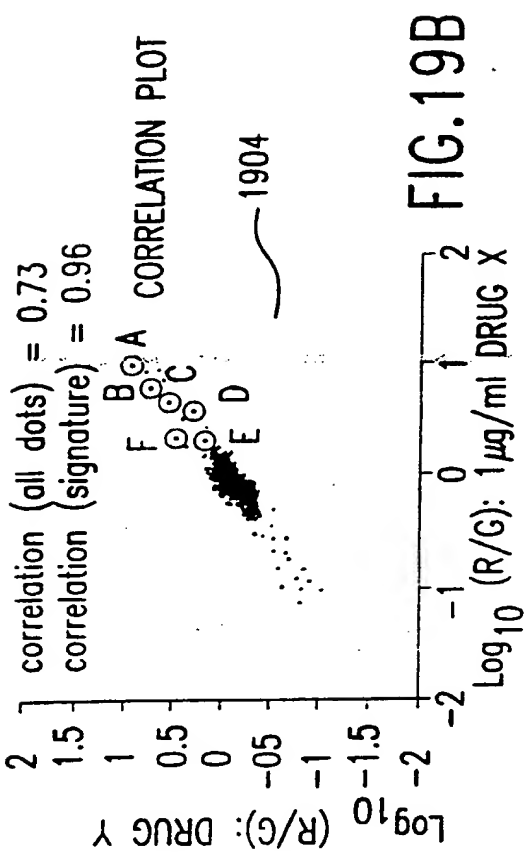



FIG.19B

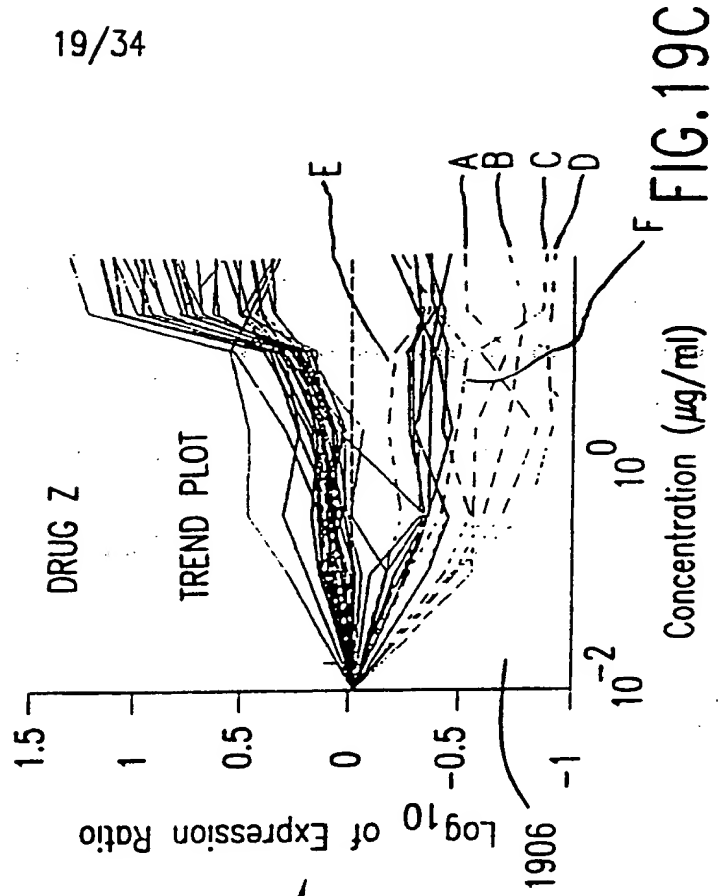



FIG.19C

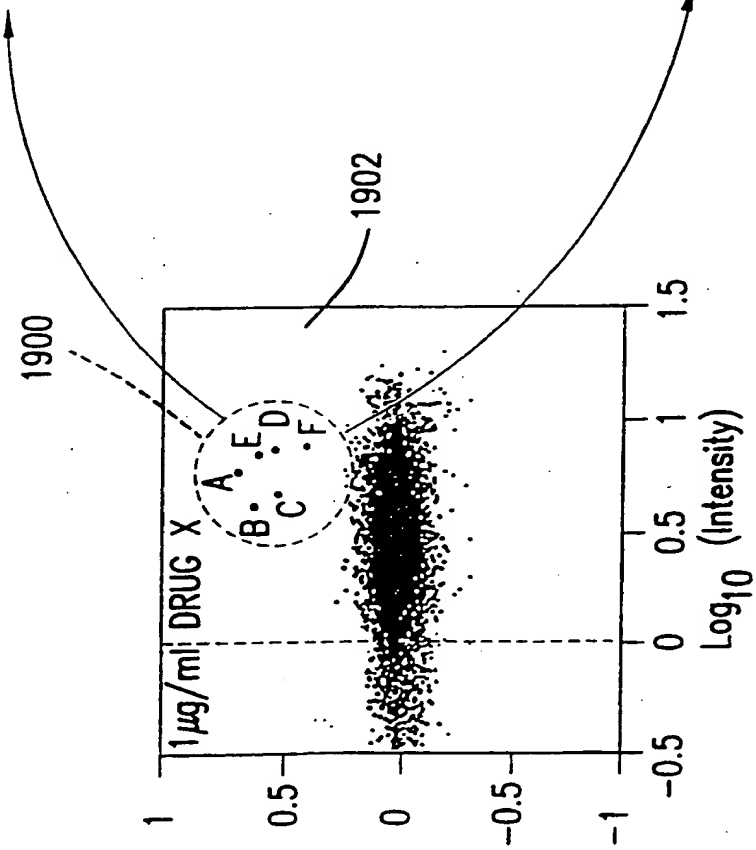



FIG.19A

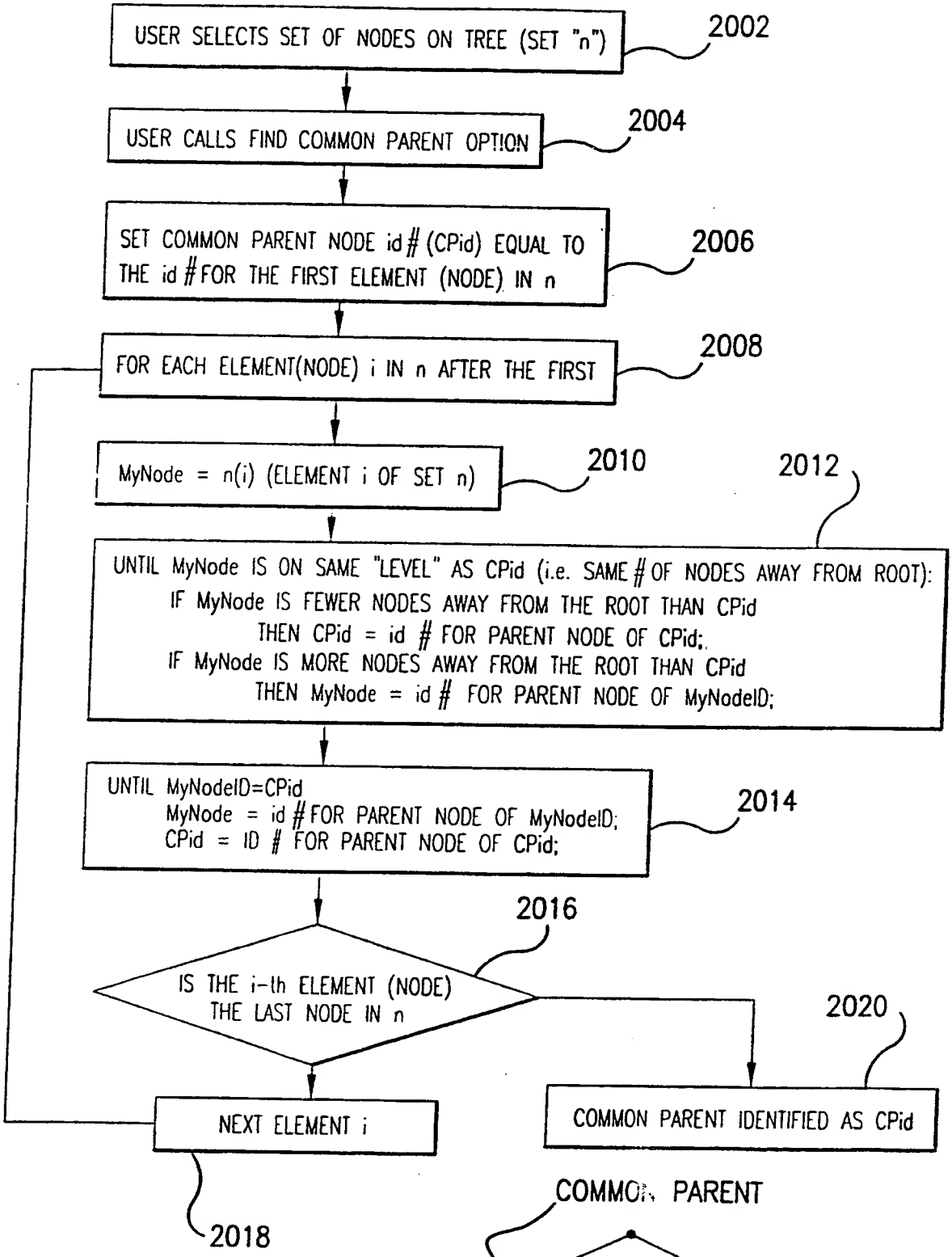



FIG.20A

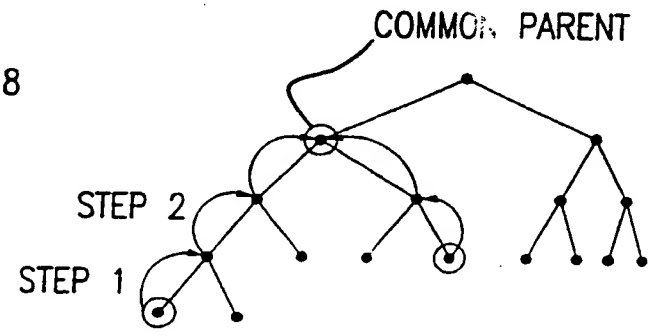



FIG.20B

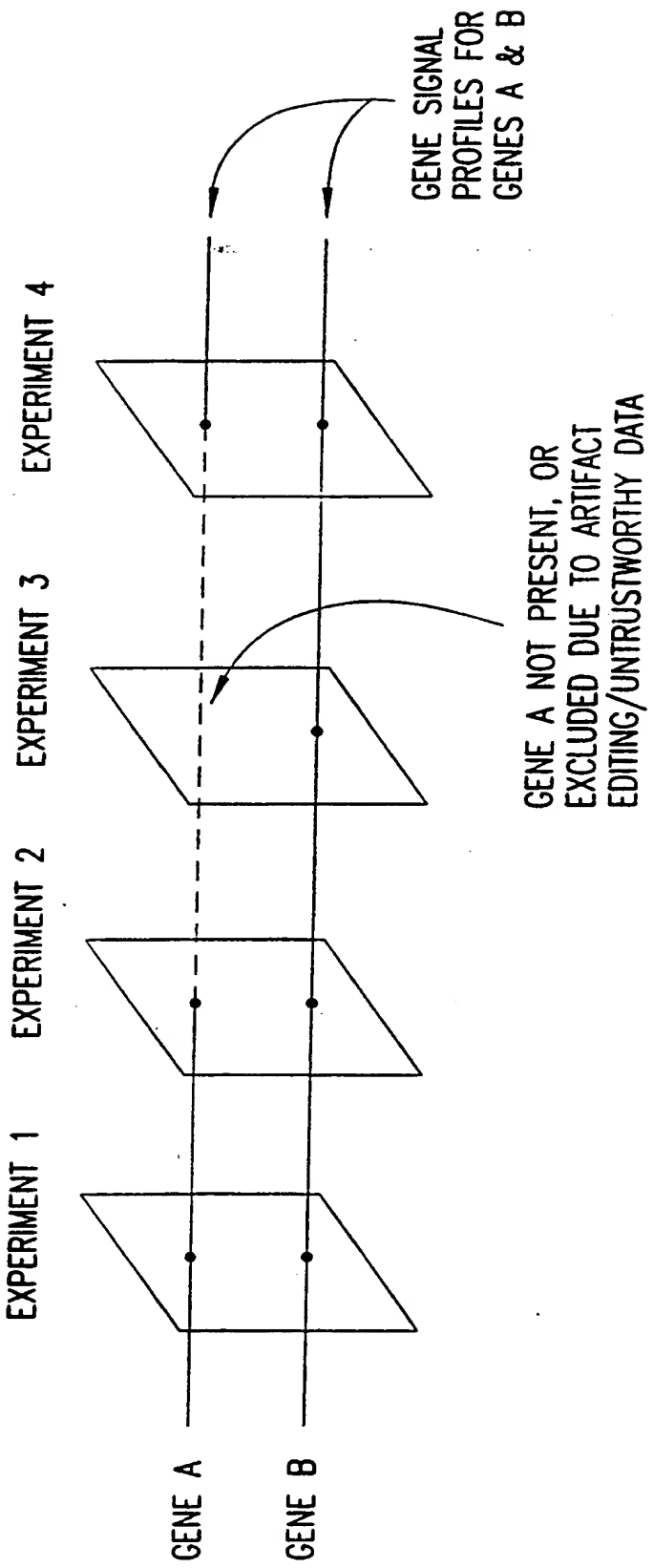



FIG.21

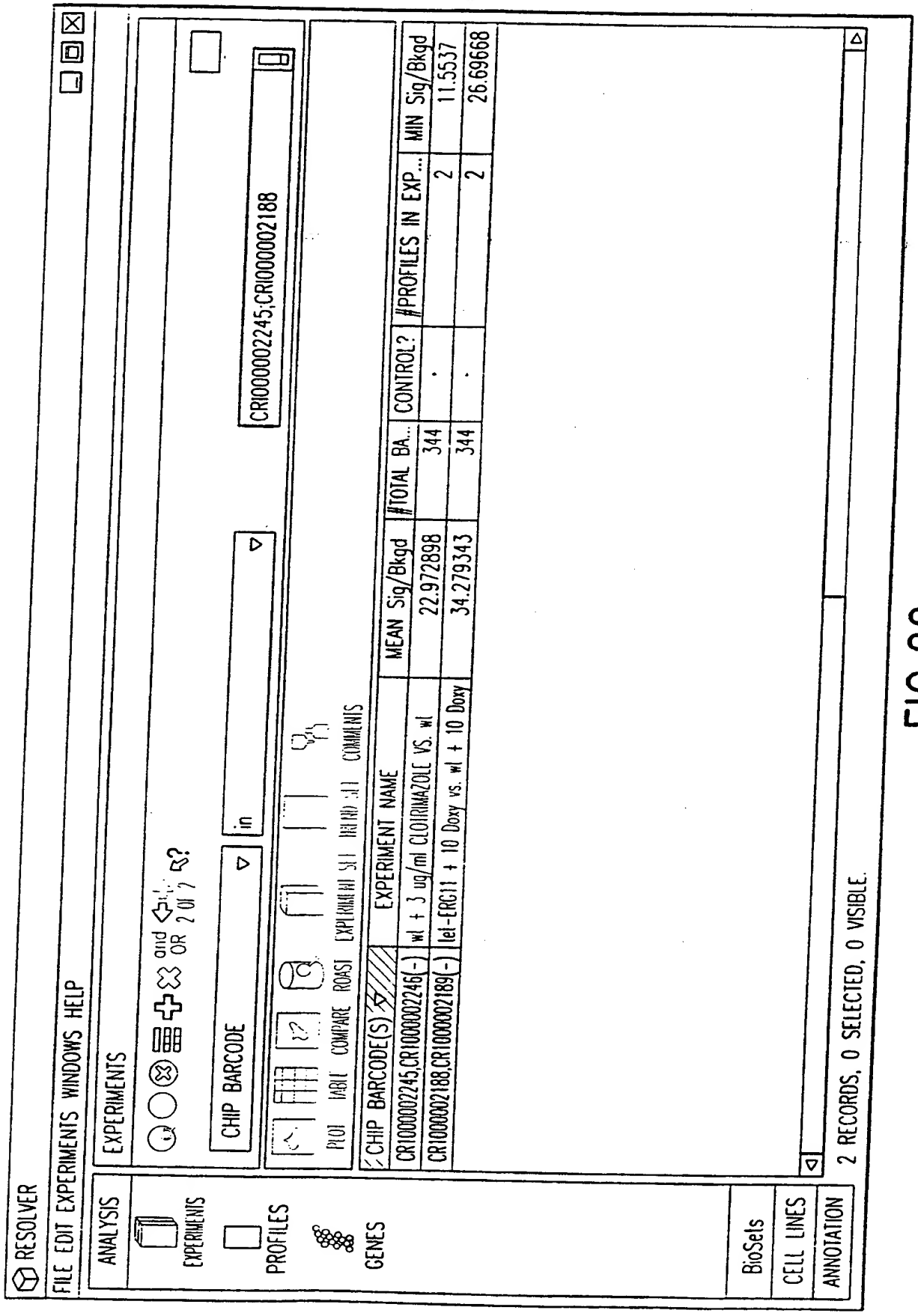



FIG.22

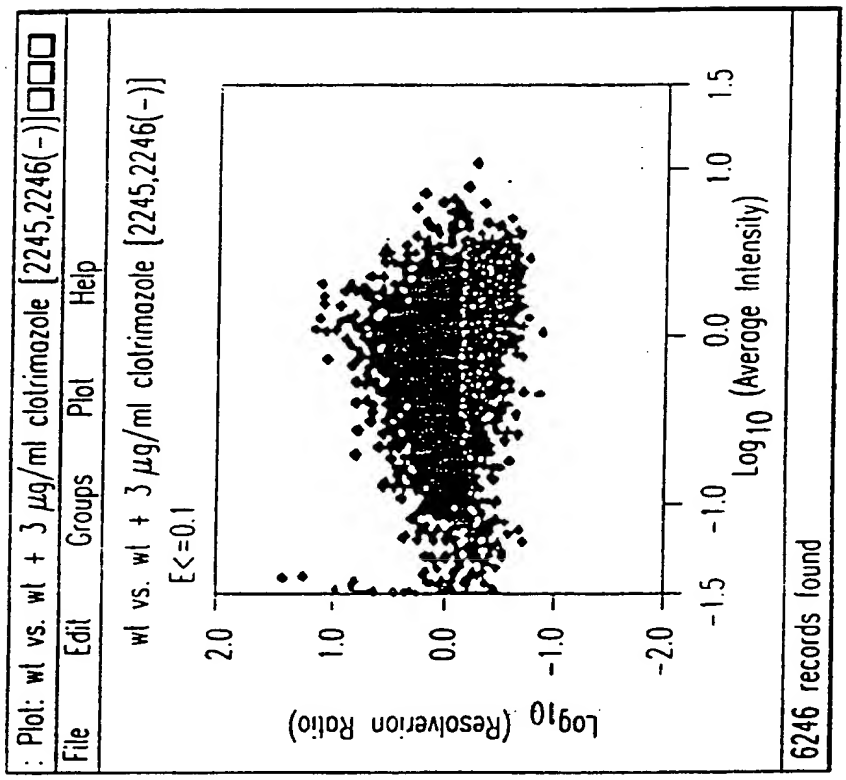



FIG.23A

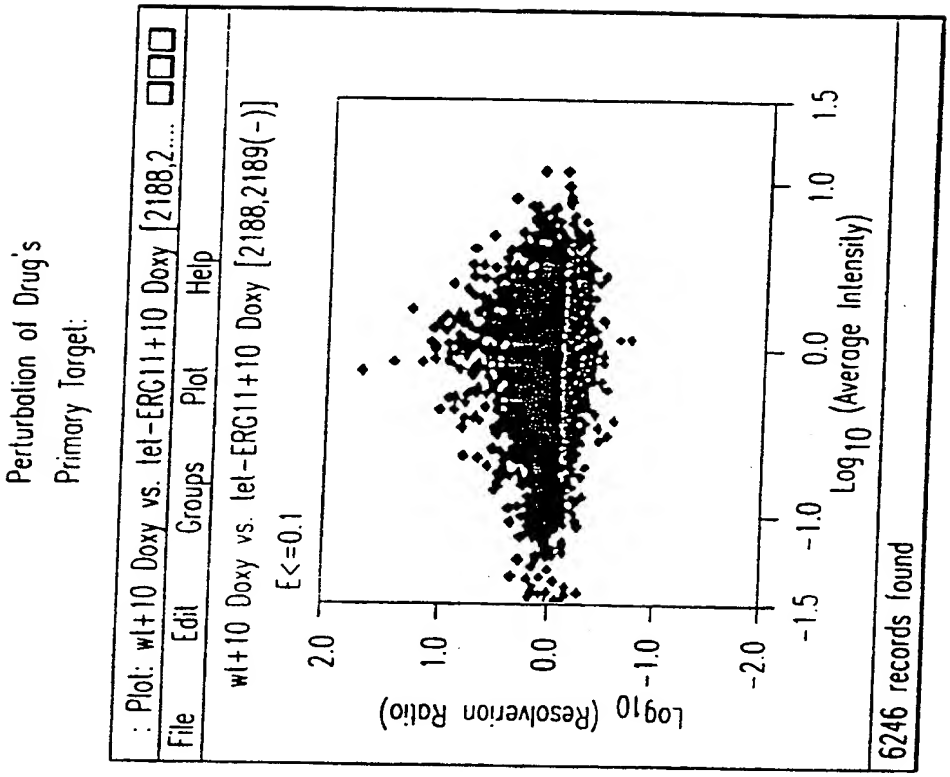



FIG.23B

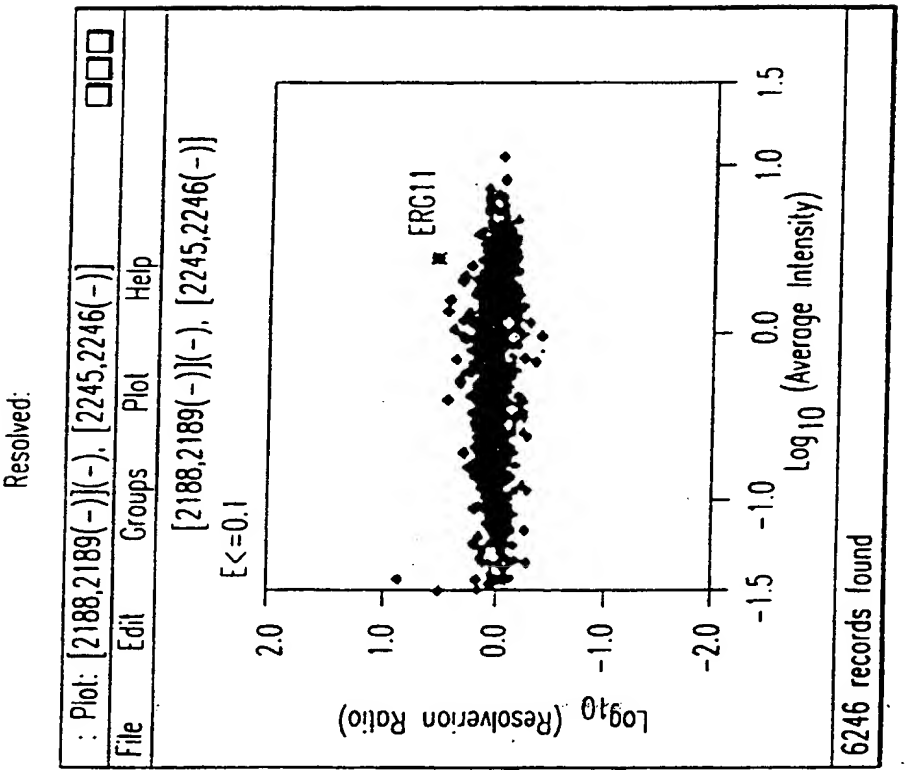



FIG.23C

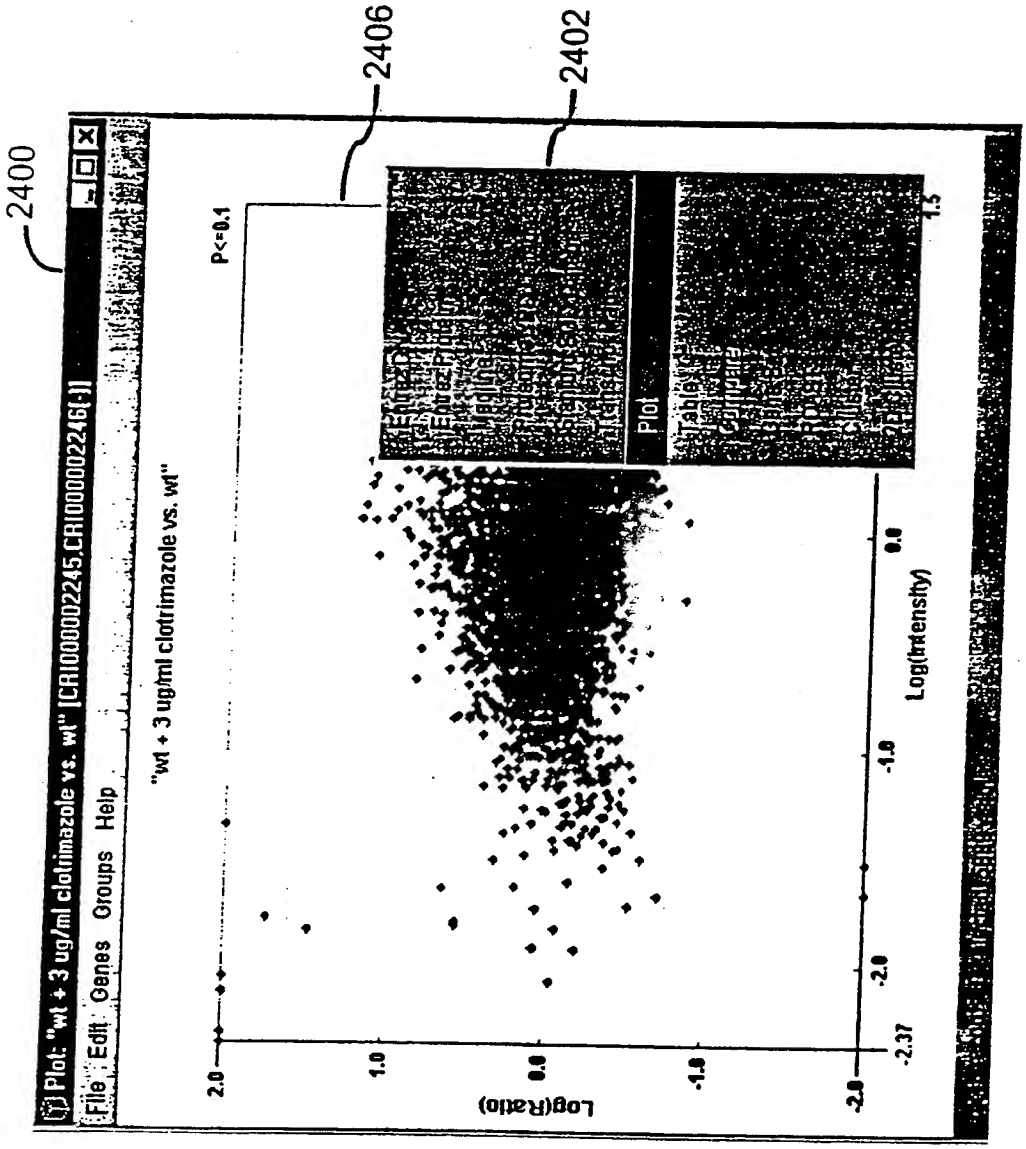



FIG.24

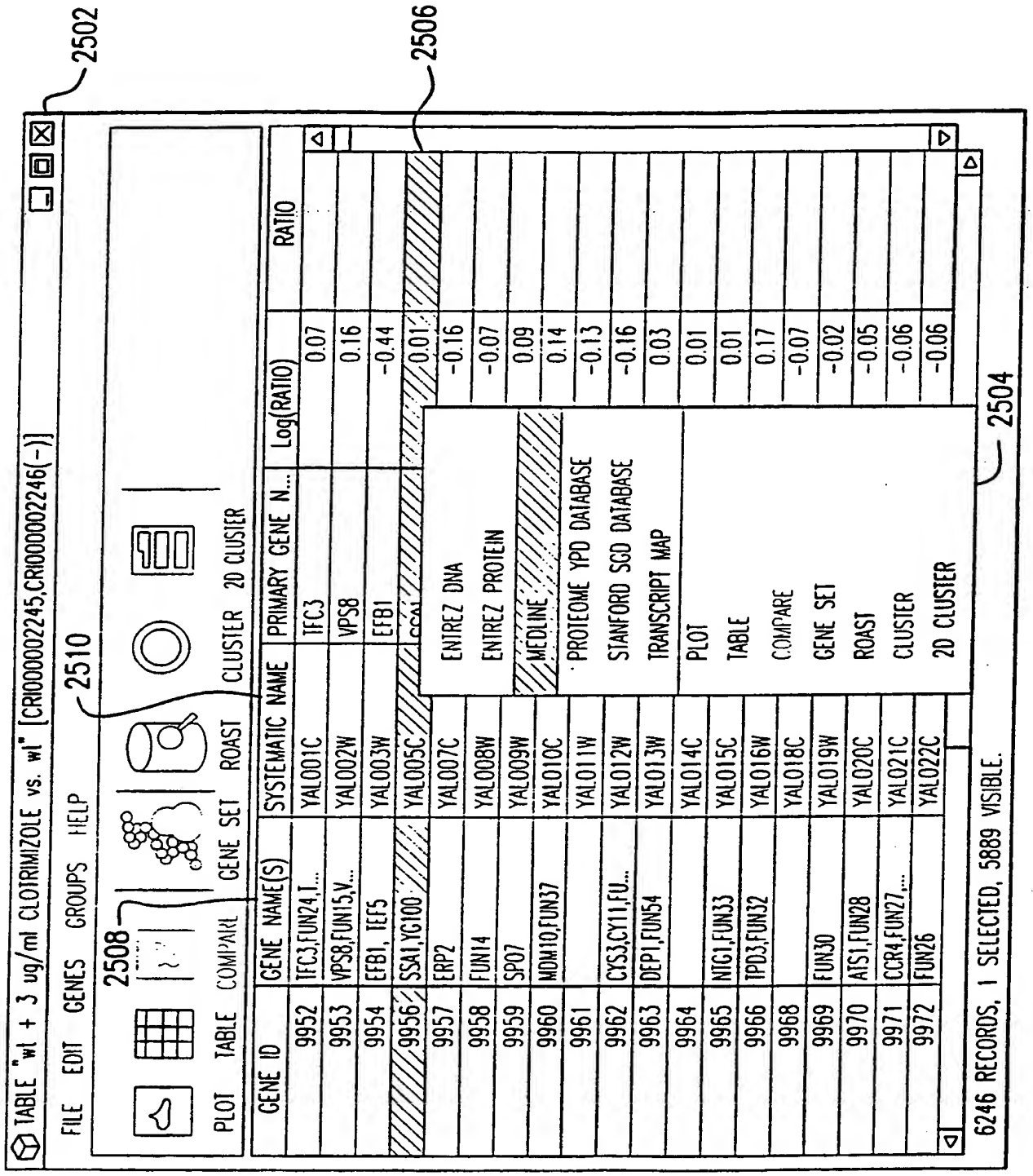



FIG.25

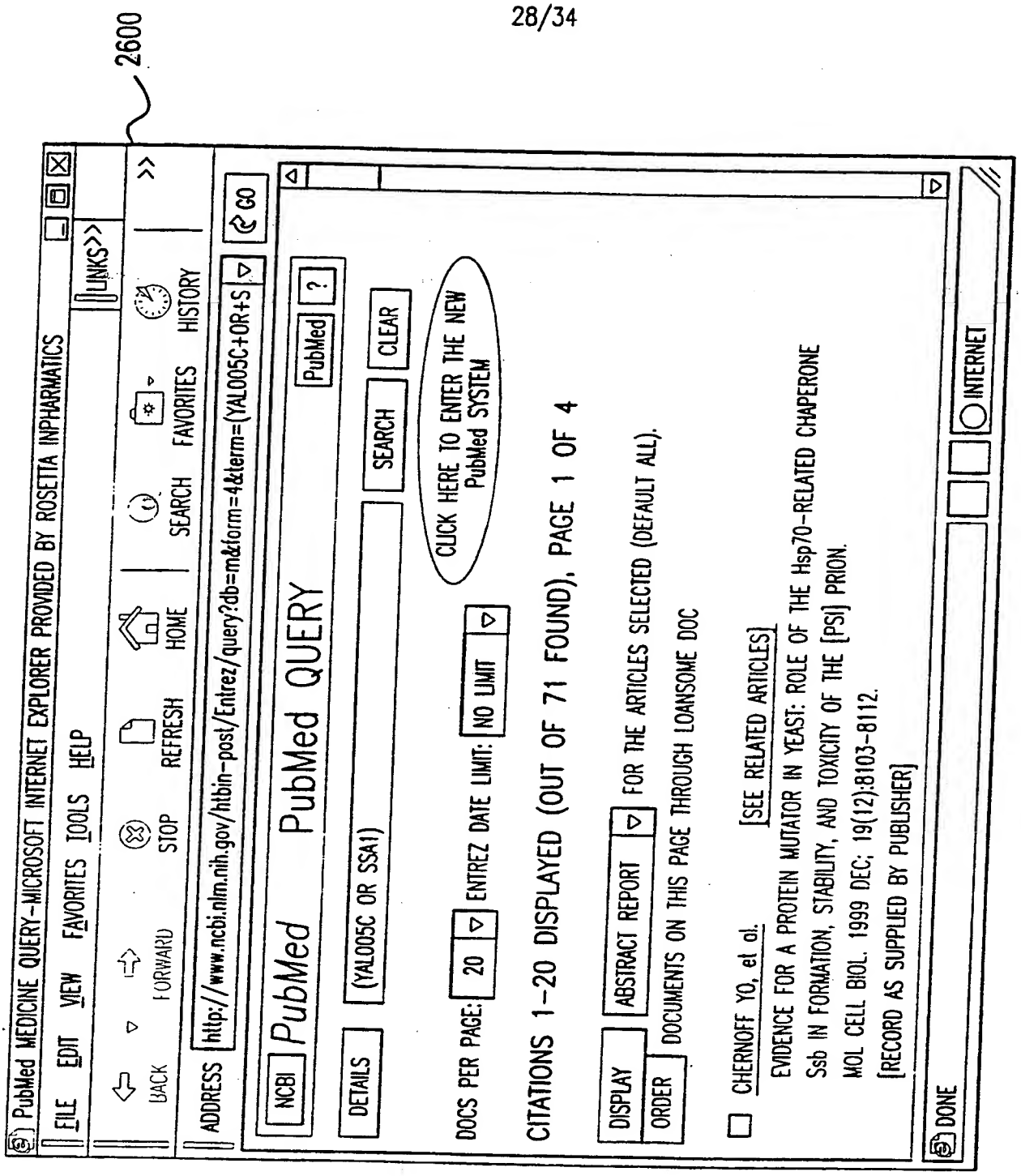



FIG.26

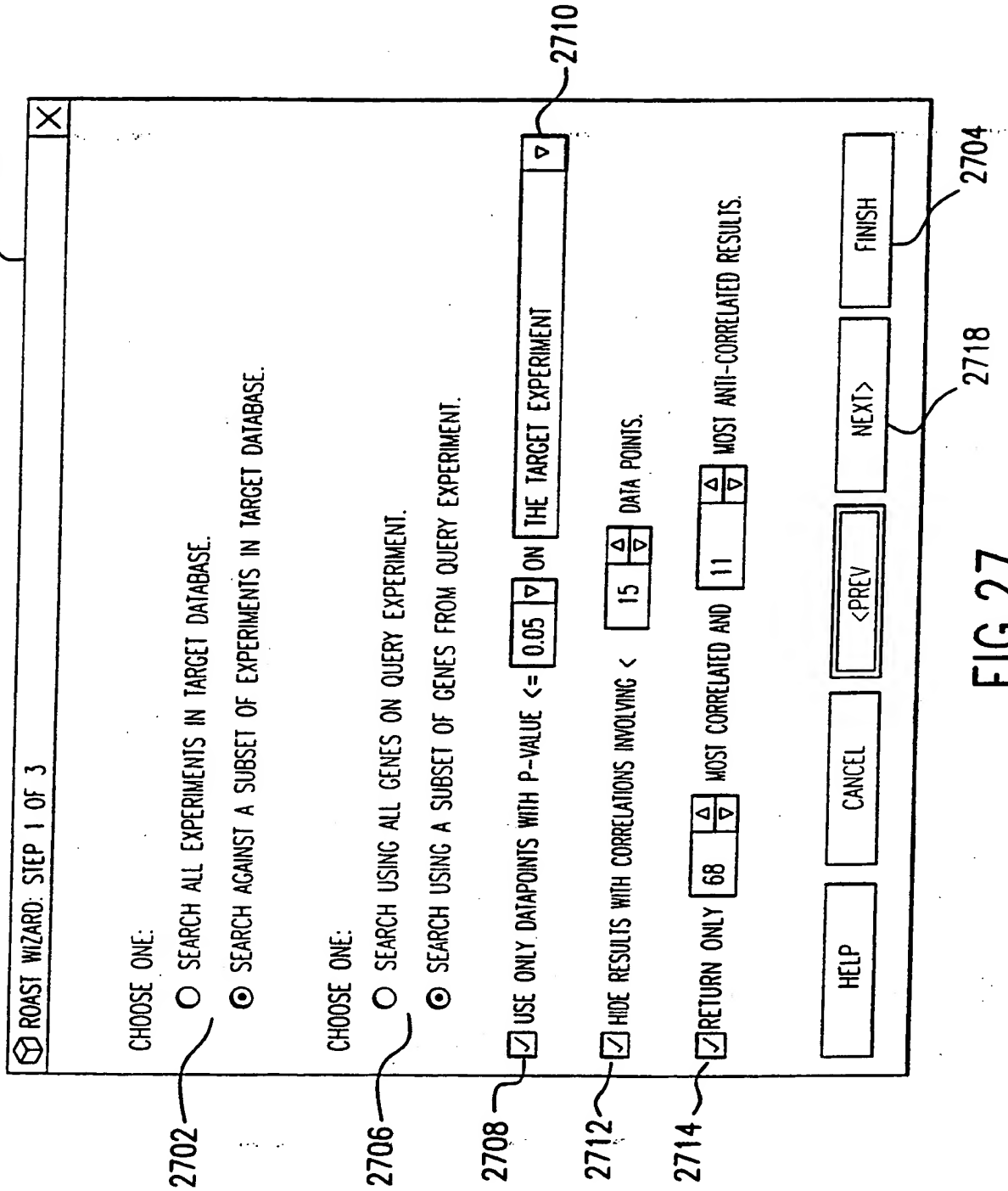



FIG.27

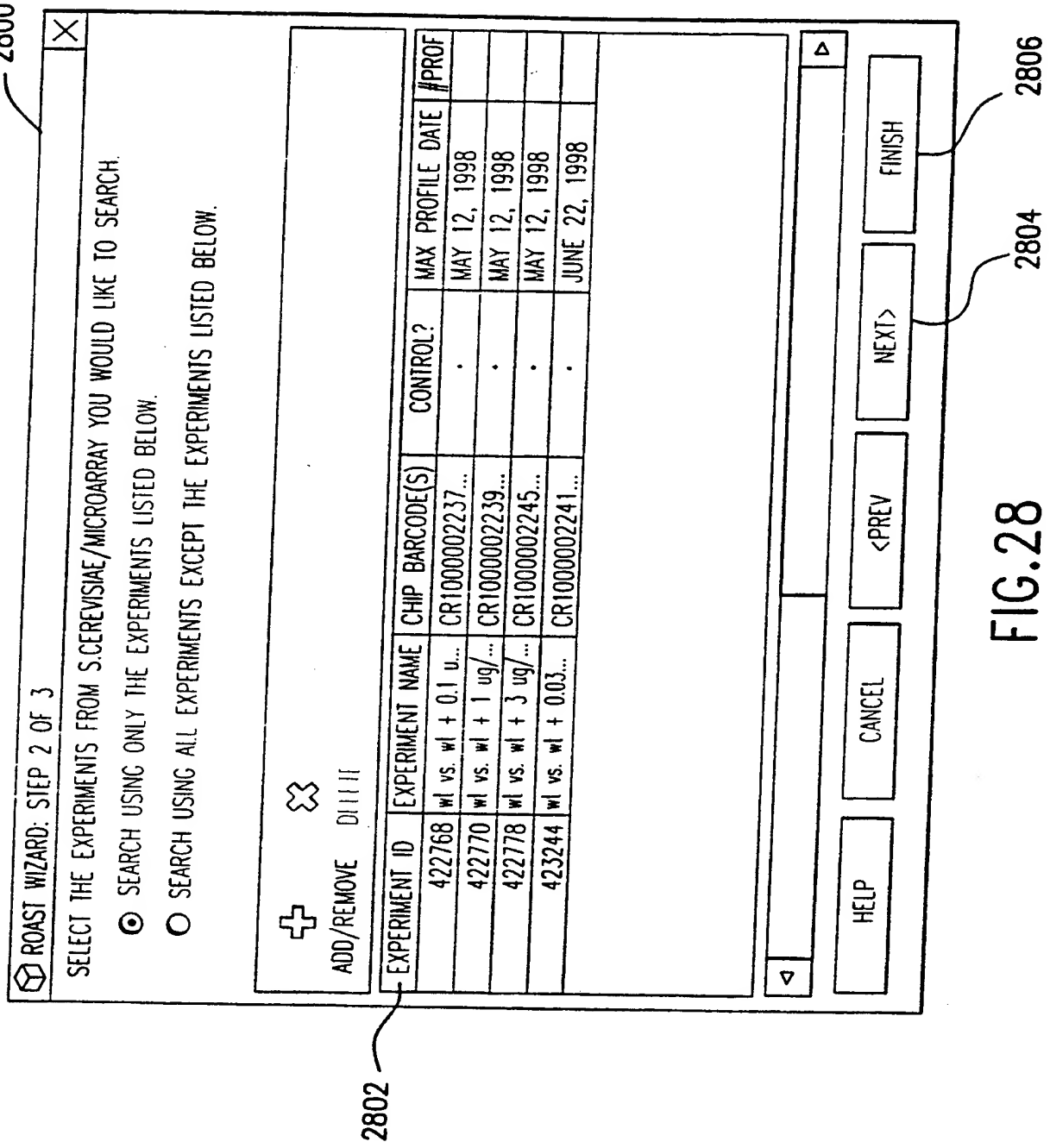



FIG.28

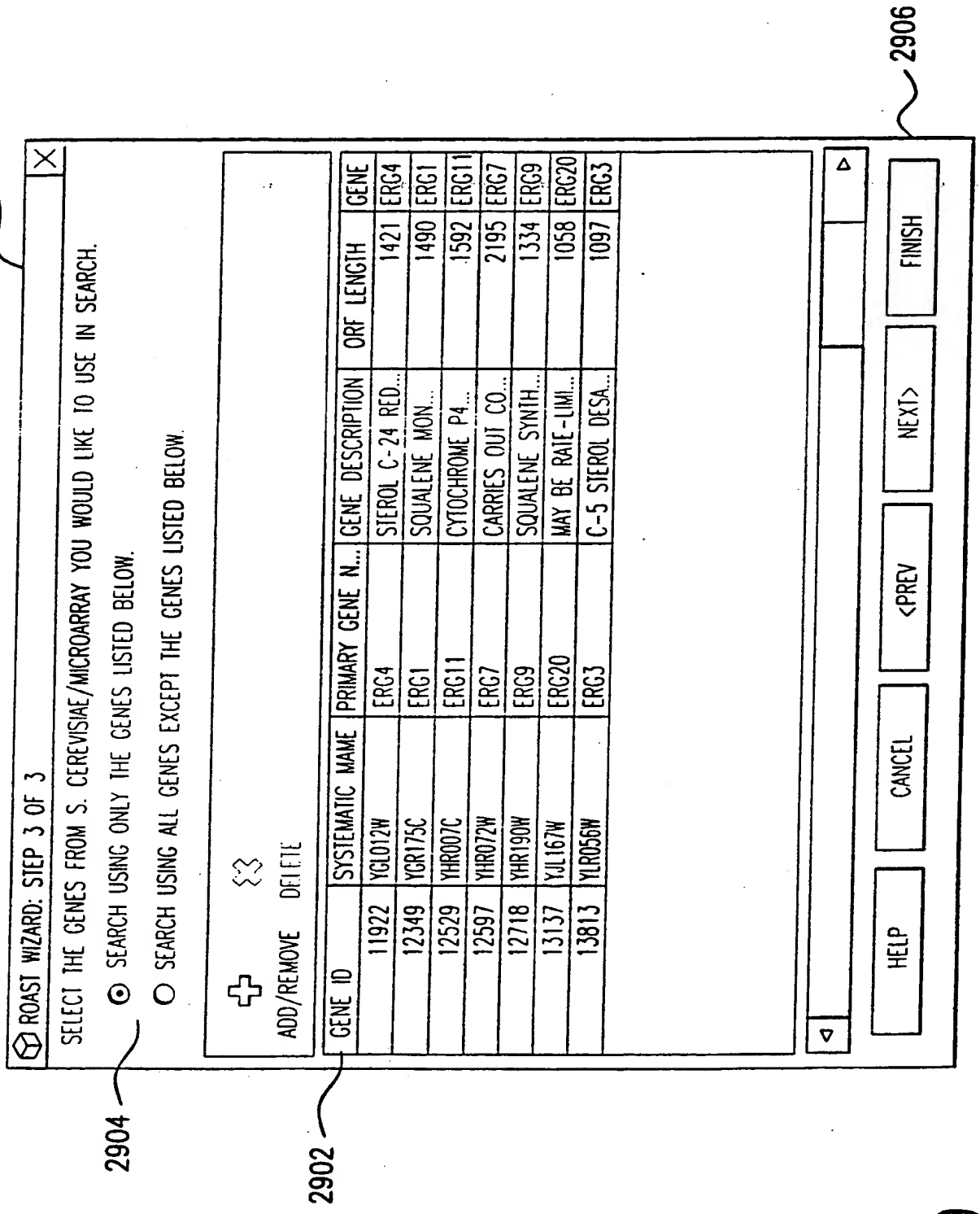



FIG.29

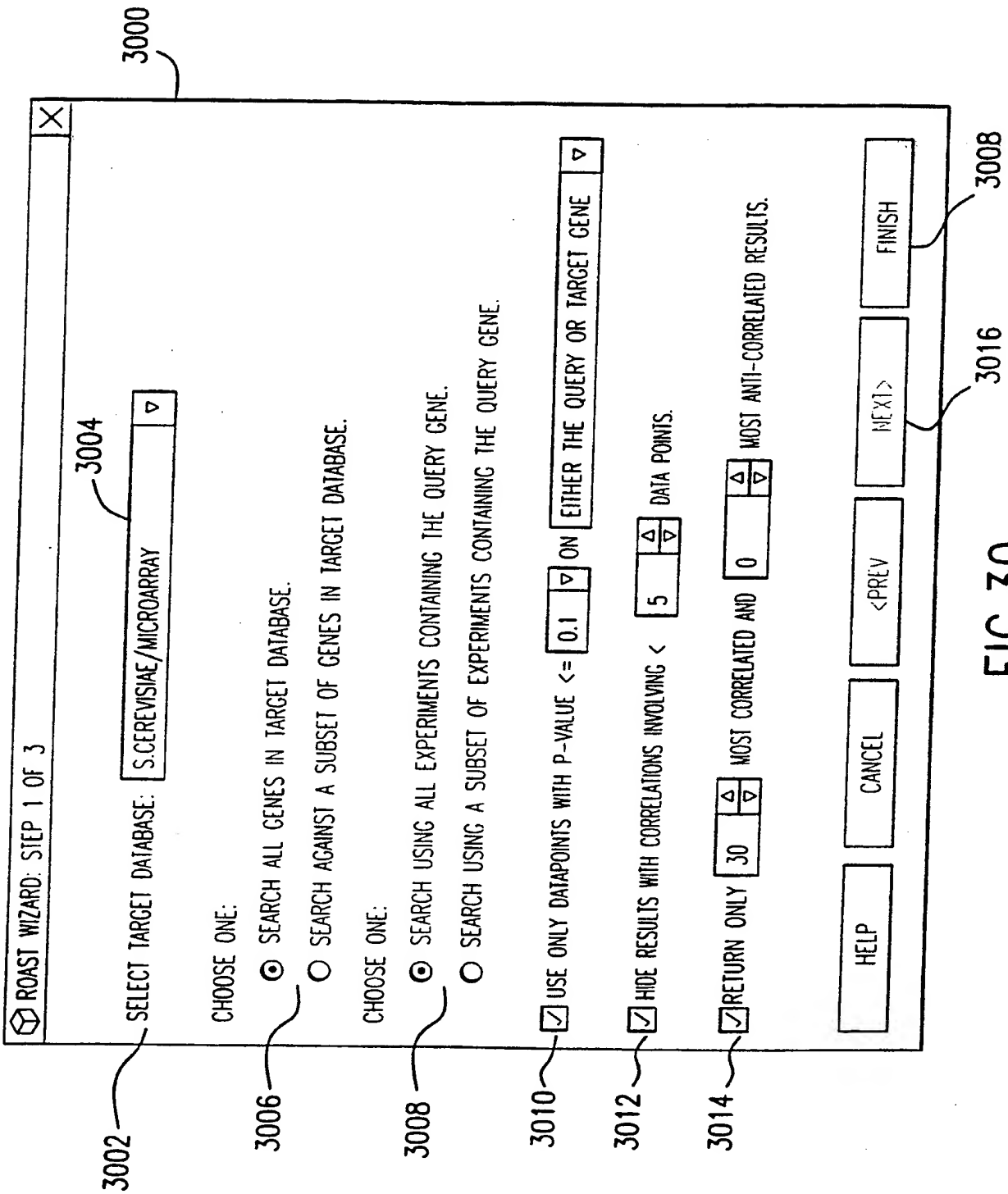



FIG.30

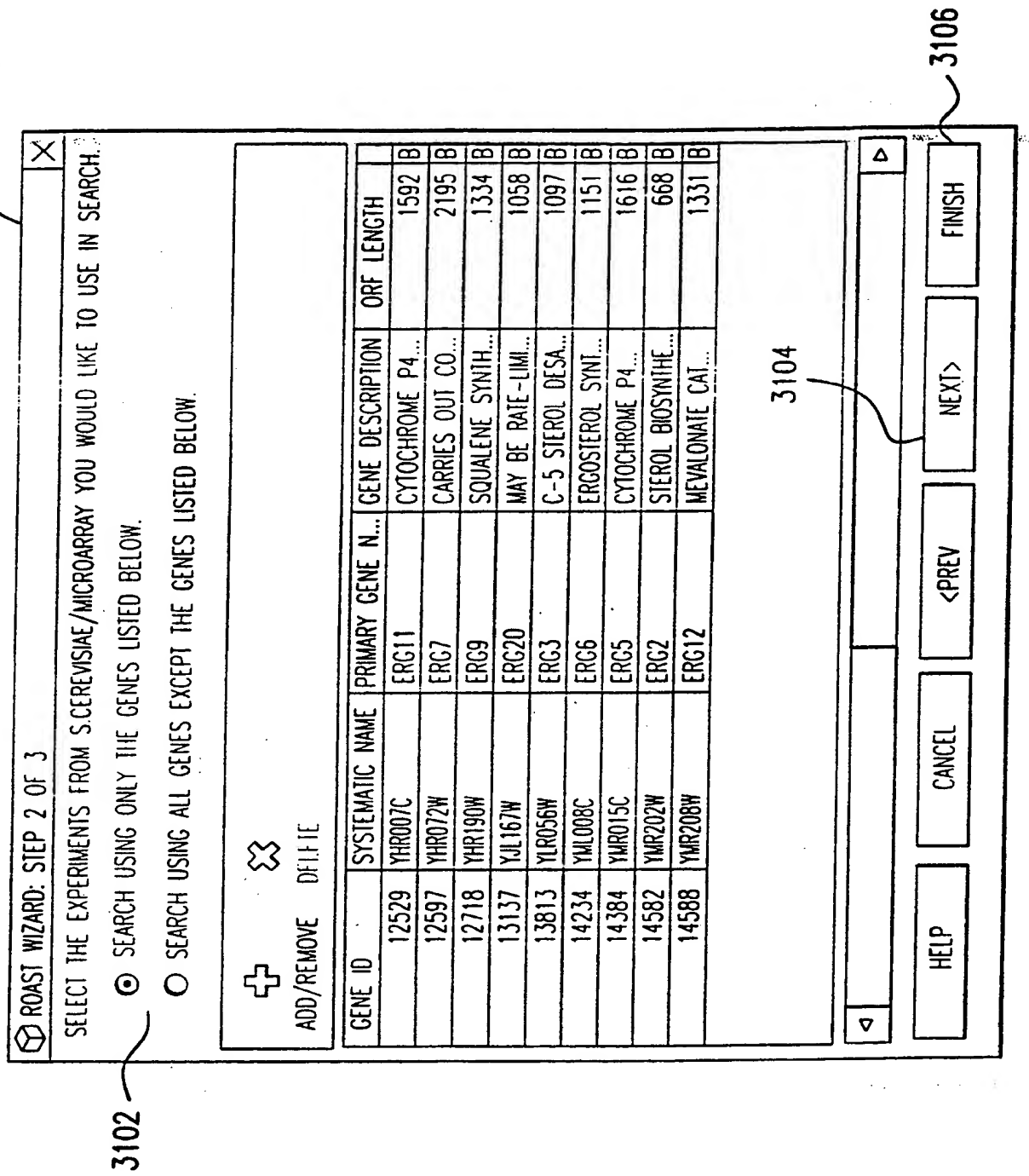



FIG.31

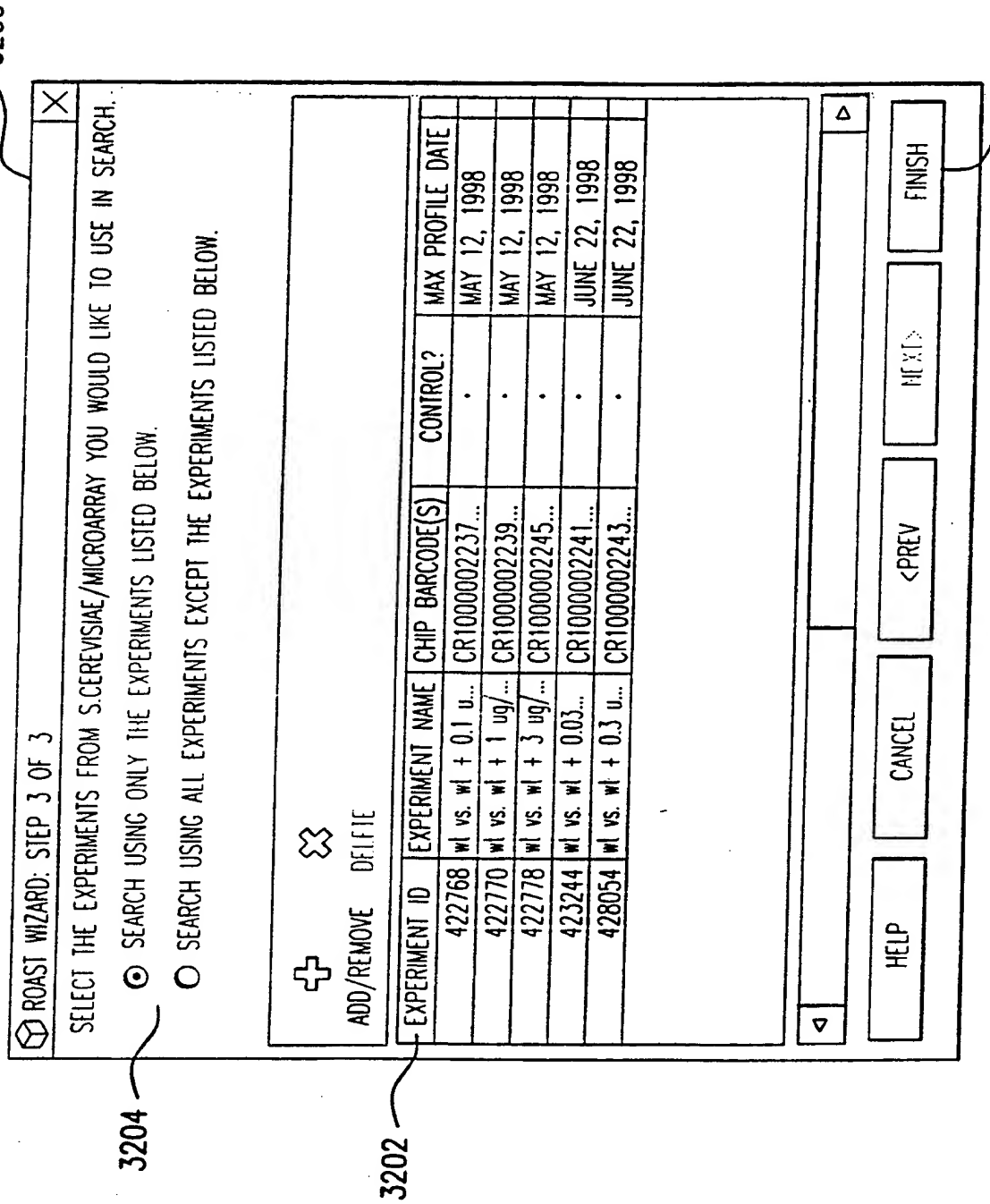



FIG.32

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30643

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/68; G06F 17/00, 7/00, 17/30
US CL : 435/6; 395/614; 707/104, 10

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6; 395/614; 707/104, 10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y,P | US 5,966,712 A (SABATINI et al.) 12 October 1999 (12.10.1999), Figures 1-5, columns 1-14. | 1-31, 34-75 |
| Y,P | US 5,970,500 A (SABATINI et al) 19 October 1999 (19.10.1999), entire document. | 1-31, 34-75 |
| Y | US 5,630,125 A (ZELLWEGER) 13 May 1997 (13.05.1997), all the figures, columns 1-15. | 1-31, 34-75 |
| A | ZHAO et al. High-density cDNA filter analysis: a novel approach for large-scale, quantitative analysis of gene expression. Gene. 1995, Vol. 156, pages 207-213. | 32-33 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"E" earlier application or patent published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 February 2000 (24.02.2000)

Date of mailing of the international search report
04 APR 2000

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Telephone No. 703-308-0196

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| International application No. |
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| PCT/US99/30643 |

**Continuation of B. FIELDS SEARCHED Item 3:** BIOCOMMERCE, CEN EMBASE, JICST-EPLUS, NTIS, PHIN, PROMT, USPATFULL, INVESTEXT, NLDB, WEST (USPT, JPAB, EPAB, TDBD, DWPI)